EXHIBIT 18

Element Biosciences

Teton™ CytoProfiling

User Guide

FOR USE WITH

AVITI24™ System, catalog # 880-00004
AVITI Operating Software v3.3.0 or later
Teton Fixed Panel Kits
Teton Custom Add-On Protein Panel Assembly Kit
Teton Optimization Kit

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CHAPTER 1

Overview

A cytoprofiling run on the AVITI24 System performs avidite base chemistry (ABC) sequencing within cell samples to detect numerous cellular RNA and proteins. The workflow requires prepared cell samples and Teton consumables.

This guide provides instructions for preparing samples, assembling the flow cell, and performing a cytoprofiling run. Before initiating a run, make sure you have read the instrument overview and safety information in the *AVITI24 System User Guide (MA-00051)*.

Cytoprofiling Run Stages

The AVITI24 operating software (AVITI OS) generates a recipe based on the assay and run parameters entered during run setup. The recipe governs each stage of the cytoprofiling run. The run is complete when the recipe is executed and primary analysis is complete.

The following stages comprise a cytoprofiling run:

- **Cell Paint**—Obtains images of cell morphological features before reading barcoded targets.
- **Amplification**—Binds probes to cellular structures on and inside the cell sample and amplifies to form polonies.
- **Batches**—Reads a set of targets, including RNA and protein targets. Each batch identifies different sets of targets.

CytoProfiling Run Consumables

A cytoprofiling run on the AVITI24 System requires one each the following Teton kits:

- Teton cartridge and reagent kit
- Teton fixed panel
- Teton slide kit
- Teton flow cell assembly kit

To perform a dual flow cell run, a quantity of two of each kit is required. For a list of kits with catalog numbers, see *Consumables and Tools* on page 42.

Teton Fixed Panels

Each fixed panel kit includes one 350-plex RNA panel and one 50-plex protein panel. The RNA and protein panels are provided in tubes that are designed to load directly onto the Teton cartridge.

The following Teton fixed panel kits are available for the AVITI24 System:

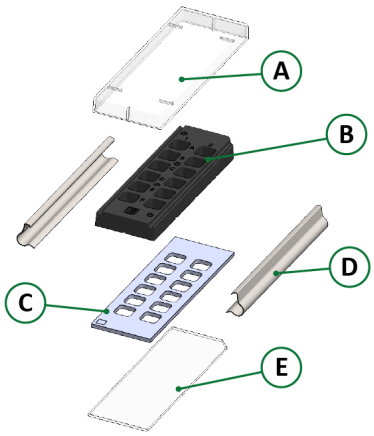
- Human MAPK-Cell Cycle Panel Kit
- Human MAPK-Apoptosis Panel Kit
- Human Neuro Panel Kit
- Human Immuno Panel Kit

For a list of targets associated with each fixed panel, see the following documentation:

- *MAPK RNA and Protein Targets Reference (MA-00062)*
- *Neuro Panel RNA and Protein Targets Reference (MA-00071)*
- *Immuno Panel RNA and Protein Targets Reference (MA-00072)*

Teton Slide Kit

The Teton Slide Kit is used for culturing cell samples directly onto the slide. The slide kit includes a glass slide with a barcode for tracking and validation, a frame, a gasket, two side clips, and a lid. The frame and the gasket determine the number of wells on the slide. Before starting a cytoprofiling run, the slide is reassembled as a flow cell with parts from the Teton Flow Cell Assembly Kit.

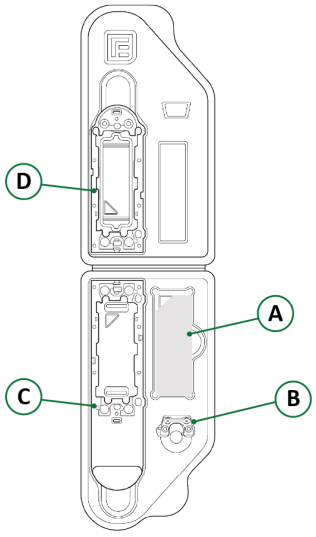


A Lid
B Frame
C Gasket
D Side clip
E Glass slide

Slide kits are available in either 12-well or 1-well and PLL-coated or uncoated combinations. Uncoated slide kits provide the option of applying a custom surface specific to your cell line. See *Custom Surface Coatings* on page 8.

Teton Flow Cell Assembly Kit

The Teton flow cell assembly kit contains an adhesive slide, two flow cell gaskets, and the top and bottom cartridge parts. After samples are prepared on the slide kit, the slide kit is disassembled. The glass slide containing the sample is affixed to the adhesive slide provided in the Teton flow cell assembly kit using the Teton flow cell assembly tools. See *Assemble the Teton Flow Cell* on page 22.



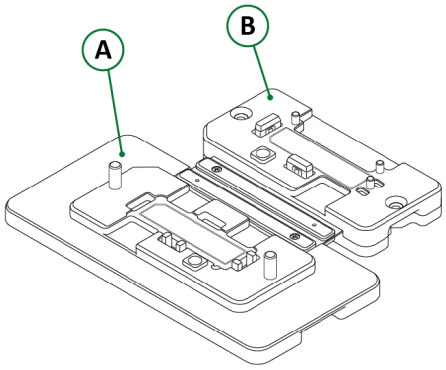
A Adhesive slide
B Flow cell gaskets (2)
C Cartridge bottom
D Cartridge top

Flow cell assembly kits are available in either 12-well or 1-well configurations. One flow cell assembly kit is required to load one sample slide for a cytoprofiling run.

Teton Flow Cell Assembly Tools

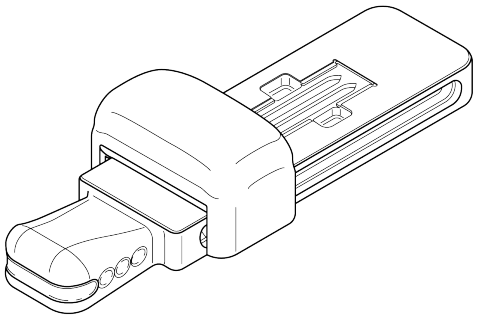
After samples are prepared using the Teton slide kit, the slide is removed and combined with parts provided in the flow cell assembly kit. The process of assembling the flow cell requires the Teton flow cell aligner and the Teton flow cell sealer.

- **Teton flow cell aligner**—The flow cell aligner aligns and adheres the sample slide from the slide kit to the adhesive slide provided in the flow cell assembly kit. One half of the aligner is labeled **Sample** and intended for the sample slide. The other half is labeled **Adhesive** and intended for the adhesive slide. After each slide is locked in place, the hinged fixture is designed to flip the Adhesive side against the Sample side, affixing the slides together. See *Align and Seal the Slides* on page 23.



A Side labeled **Sample** for sample slide
B Side labeled **Adhesive** for adhesive slide

- **Teton flow cell sealer**—The flow cell sealer ensures a secure seal of the two affixed slides as you *slowly* move the roller grip forward and back. For more information, see *Caring for the Teton Flow Cell Sealer* on page 46.



Teton Custom Add-On Protein Panel Assembly Kit

The Teton Custom Add-On Protein Panel Assembly Kit, catalog # 860-00036, enables up to 88 additional protein targets of your choosing as a spike-in to a Teton fixed panel kit. For more information, see *Teton Custom Add-On Protein Panel* on page 34.

Teton Optimization Kit

The Teton Optimization Kit, catalog # 860-00022, enables an early assessment of the best conditions for custom surface coating, cell culture technique, seeding densities, and growth for cell lines on a Teton slide kit. The kit includes a 12-well flow cell assembly kit and optimization kit reagents. For more information, see *Teton Optimization Kit* on page 37.

CHAPTER 2

Custom Surface Coatings

Uncoated Teton slide kits are available for preparing a user-applied custom coating. Applying a coating increases cell adhesion and avoids cell loss during washing and other assay steps. If you are using a PLL-coated slide kit, a custom surface is not necessary.

Slide Preparation

Regardless of your preferred custom coating, the slide preparation steps are the same. Use the following instructions to prepare the slide and then proceed to the preferred surface coating instructions.

NOTE
Perform all steps in a biosafety cabinet.

1. Gather the following consumables:
   - 0.1 N NaOH solution
   - Biological-grade/RNase-free water
   - Teton Slide Kit
2. Add the appropriate volume of 0.1 N NaOH solution to each well. Make sure the entire surface in each well is covered.
   - 12-well slide—150 µl
   - 1-well slide—2 ml
3. Incubate at room temperature for 15 minutes inside the biosafety cabinet.
4. Pipette to remove the solution from each well.
5. Wash each well with the appropriate volume of biological-grade/RNase-free water.
   - 12-well slide—200 µl
   - 1-well slide—3 ml
6. Repeat the wash 5 more times. Pipette to remove the final wash solution from each well.
7. Proceed to the preparation steps for your preferred surface coating:
   - *Collagen Coating* on page 9
   - *Fibronectin Coating* on page 9
   - *Gelatin Coating* on page 10
   - *Laminin Coating* on page 10
   - *Matrigel Coating* on page 11
   - *Poly-L-Lysine (PLL) Coating* on page 11

Collagen Coating

1. Make sure *Slide Preparation* is complete.
2. Gather the following consumables:
   - » Collagen Type 1 stock solution—Store at 2°C to 8°C (MilliporeSigma, catalog # C3867-1VL)
   - » Hydrochloric acid (HCl), 0.01 N
   - » Biological-grade/RNase-free water
3. If the collagen stock solution appears thick, set aside at room temperature for 15–30 minutes. Do not exceed 2 hours.
4. Dilute the collagen I stock solution to a concentration of 20 µg/ml with 0.01 N HCl.
5. Add the appropriate volume of diluted collagen solution to each well. Make sure the entire surface in each well is covered.
   - » 12-well slide—150 µl
   - » 1-well slide—2 ml
6. Incubate at room temperature for 1 hour inside the biosafety cabinet.
7. Carefully pipette along the wall of each well to remove remaining solution without disturbing the slide surface.
8. Wash each well with the appropriate volume of biological-grade/RNase-free water.
   - » 12-well slide—200 µl
   - » 1-well slide—3 ml
9. Repeat the wash two more times. Pipette along the wall of each well to *completely* remove the final wash solution.
10. Allow the surface to air-dry for 15 minutes.
11. Seal the wells with an adhesive seal and store the slide dry at 2°C to 8°C for up to 10 days. Do not use if surface cracking exists.

Fibronectin Coating

1. Make sure *Slide Preparation* is complete.
2. Gather the following consumables:
   - » Fibronectin stock solution—Store at 2°C to 8°C (MilliporeSigma, catalog # F1141-2MG)
   - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
   - » Biological-grade/RNase-free water
3. Dissolve and dilute the fibronectin stock solution to a final concentration of 2 µg/ml in 1X PBS.
4. Add the appropriate volume of diluted fibronectin solution to each well. Make sure the entire surface in each well is covered.
   - » 12-well slide—150 µl
   - » 1-well slide—2 ml
5. Incubate at room temperature for 1 hour inside the biosafety cabinet.
6. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
7. Wash each well with the appropriate volume of biological-grade/RNase-free water.
   - » 12-well slide—200 µl
   - » 1-well slide—3 ml
8. Repeat the wash two more times. Pipette along the wall of each well to *completely* remove the final wash solution.
9. Allow the surface to air-dry for 15 minutes.
10. Seal the wells with an adhesive seal and store the slide dry at 2°C to 8°C for up to 10 days. Do not use if surface cracking exists.

Gelatin Coating

1. Make sure *Slide Preparation* is complete.
2. Gather the following consumables:
   - » Gelatin solution, Type B, 2% in H_2O—Store at 2°C to 8°C (MilliporeSigma, catalog # G1393-20ML)
   - » Biological-grade/RNase-free water
3. If the gelatin stock solution is cloudy, place the gelatin in a 37°C water bath for 1 hour or until the solution is clear. Do not exceed 2 hours.
4. Dissolve and dilute the gelatin stock solution to a final concentration of 50 µg/ml in biological-grade/RNase-free water.
5. Add the appropriate volume of diluted gelatin solution to each well. Tap or swirl the slide kit to ensure coverage in each well..
   - » 12-well slide—80 µl
   - » 1-well slide—1 ml
6. Incubate at room temperature for 1 hour inside the biosafety cabinet.
7. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
8. Wash each well with the appropriate volume of biological-grade/RNase-free water.
   - » 12-well slide—150 µl
   - » 1-well slide—2 ml
9. Repeat the wash two more times. Pipette along the wall of each well to remove the final wash solution.
10. Add the appropriate volume of biological-grade/RNase-free water and seal the wells with an adhesive seal. Do not allow the surface to dry.
    - » 12-well slide—150 µl
    - » 1-well slide—2 ml
11. Store the prepared slide at 2°C to 8°C for up to 10 days. Do not use if discoloration or surface cracking exists.

Laminin Coating

1. Make sure *Slide Preparation* is complete.
2. Gather the following consumables:
   - » Laminin stock solution—Store at -85°C to -75°C (Gibco Laminin Mouse Protein, Natural, catalog # 23017-015)
   - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
3. Thaw laminin at 2°C to 8°C for 15–30 minutes. Do not exceed 1 hour.
   - » Avoid rapid warming of laminin, which causes laminin to form a gel and prevents further use.
   - » Avoid multiple thaw cycles. Store small quantities of laminin at -25°C to -15°C for up to 6 months.
   - » Place the laminin in an ice bucket when handling at room temperature.
4. Dissolve and dilute laminin stock solution to a final concentration of 50 µg/ml in 1X PBS.
5. Add the appropriate volume of diluted laminin solution to each well. Tap or swirl the slide kit to ensure coverage in each well.
   - » 12-well slide—80 µl
   - » 1-well slide—1 ml
6. Incubate at room temperature for 1 hour inside the biosafety cabinet.
7. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
8. Wash each well with the appropriate volume of 1X PBS.

- 12-well slide—150 µl
- 1-well slide—2 ml

9. Repeat the wash two more times.
10. Add the appropriate volume of 1X PBS and seal the wells with an adhesive seal. Do not allow the surface to dry.
    - 12-well slide—150 µl
    - 1-well slide—2 ml
11. Store the slide at 2°C to 8°C for up to 10 days. Do not use if discoloration or surface cracking exists.

Matrigel Coating

1. Make sure *Slide Preparation* is complete.
2. Gather the following consumables:
    - Matrigel stock solution—Store at -25°C to -15°C (Corning Matrigel Basement Membrane Matrix, catalog # 356237)
    - 1X Phosphate Buffered Saline (PBS), pH 7–7.4, chilled
3. Thaw matrigel at 2°C to 8°C for 1 hour or until it liquifies and appears less viscous.
    - Avoid multiple thaw cycles. Store small quantities of matrigel at -25°C to -15°C for up to 2 years.
    - When thawed, swirl the vial of matrigel to ensure all the material is dispersed.
4. If not already chilled, chill the 1X PBS at 2°C to 8°C for 30 minutes.
5. Dissolve and dilute matrigel stock solution to a final concentration of 0.1–0.25 mg/ml in chilled 1X PBS.
    - Optimize the matrigel concentration for your cell line.
    - Place the matrigel in an ice bucket when handling at room temperature.
6. Place the slide kit on ice to help spread the matrigel solution in the wells in the next step.
7. Add the appropriate volume of diluted matrigel solution to each well. Tap or swirl the slide kit to ensure coverage in each well.
    - 12-well slide—80 µl
    - 1-well slide—1 ml
8. Incubate at room temperature for 1 hour inside the biosafety cabinet.
9. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
10. Add the appropriate volume of 1X PBS and seal the wells with an adhesive seal. Do not allow the surface to dry.
    - 12-well slide—150 µl
    - 1-well slide—2 ml
11. Store the slide at 2°C to 8°C for up to 10 days. Do not use if discoloration or surface cracking exists.

Poly-L-Lysine (PLL) Coating

Teton slide kits are available with a PLL-coating. For a user-applied PLL coating on an uncoated slide kit, use the following instructions.

1. Make sure *Slide Preparation* is complete.
2. Gather the following consumables:
    - PLL stock solution, 0.01%—Store at 2°C to 8°C (MilliporeSigma, catalog # P4707-50ML)
    - Biological-grade/RNase-free water
3. Add the appropriate volume of 0.01% PLL solution to each well. Make sure the entire surface in each well is covered.
    - 12-well slide—150 µl
    - 1-well slide—2 ml

4. Incubate at room temperature for 15 minutes inside the biosafety cabinet.
5. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
6. Wash each well with the appropriate volume of biological-grade/RNase-free water.
   - » 12-well slide—200 µl
   - » 1-well slide—3 ml
7. Repeat the wash two more times. Vacuum aspirate along the wall of each well to *completely* remove the final wash solution.
8. Allow the surface to air-dry for 15 minutes.
9. Seal the wells with an adhesive seal and store the slide dry at 2°C to 8°C for up to 7 days.

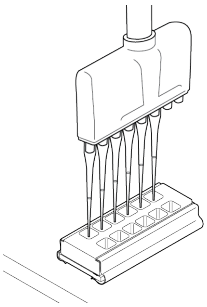
CHAPTER 3

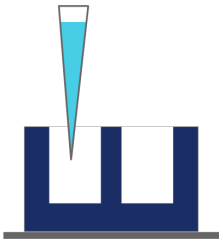
Sample Preparation

Sample preparation on a Teton slide kit is required for all Teton runs on an AVITI24 System and when using the Teton Optimization Kit. This section describes sample preparation for both adherent cells and suspension cells.

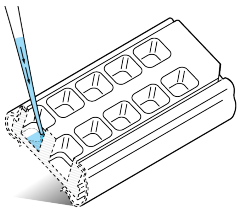
Protocol Guidelines

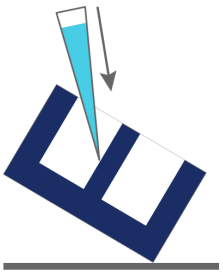
- **Perform sample preparation steps in a biosafety cabinet.** Steps involving live cells ***must*** be performed in a biosafety cabinet.
- Avoid disturbing the slide surface throughout the protocol.
- Do not allow cells to dry out. Allowing cells to dry out can result in cell detachment.
- Do not fix more than three slides at one time.
- Ensure proper pipette placement during on-flow cell treatments.



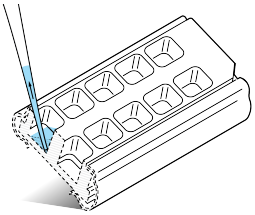


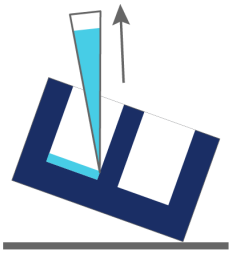
- When dispensing cells, hold the pipette perpendicular to the glass slide surface and dispense into the center of each well.
- Do not touch the slide surface.
- Do not swirl the pipette when loading.
- To reduce performance variability, ensure the slide kit is on a flat and level surface during cell seeding.





- When adding liquid, slowly dispense along the middle of the well wall.
- Do not make contact with the slide surface.
- Dispense slowly to reduce force of the liquid onto the slide surface.





- When removing liquid, position the pipette tip in the corner of the well.
- Do not make contact with the slide surface.

Culture and Fix Adherent Cells

Sample preparation of adherent cells involves steps to culture cells on the Teton slide kit and then fix cultured cells.

- **Culture**—The culture step seeds freshly dissociated cells onto the treated slide surface for growth and proliferation, resulting in a consistent cell layer.
- **Fix**—The fixation step binds cells to the slide while halting cell function and preserving the structure of the bound cells.

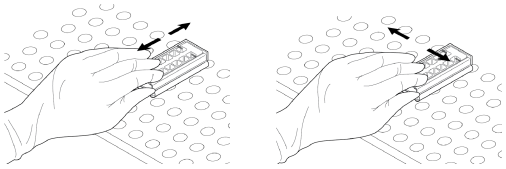
Culture Adherent Cells

1. Gather the following consumables:
   - » Cell culture medium appropriate for the cell line
   - » Teton Slide Kit
2. Warm the cell culture medium in a 37°C water bath.
3. If you prepared a custom surface coating, pipette to remove any liquid stored in the wells.
4. Wash each well with the appropriate volume of culture medium. Slightly tip the slide kit and slowly dispense along the middle of each well wall. Do not contact the slide surface. Ensure the media covers the surface in each well.
   - » 12-well slide—200 µl
   - » 1-well slide—3 ml
5. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
6. Repeat the wash one time.
7. Ensure the cells are fully dissociated to single cells and counted.
8. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently load the appropriate volume of suspended cells in the center of the well. Do not swirl the pipette when loading. Do not disturb the surface.
   - » 12-well slide—150 µl
   - » 1-well slide—2 ml

   Use the following information to estimate initial cell seeding density.

12- well slide kit	Well size: 7 mm x 7 mm	Element uses 9,000–10,000 HeLa cells per well
1- well slide kit	Well size: 54 mm x 19 mm	Element uses 120,000–180,000 HeLa cells per well

9. Cover the slide kit. Gently distribute the cells for 30 seconds using a forward-and-back, then side-to-side motion.
   - » At a slow pace, move forward and back and side to side covering at least 5 inches (12.5 cm) in each direction. Continue to move forward and back, then side to side for a total of 1 minute.
   - » *Do not move in a circular motion*
   - » Do not allow the liquid to splash within the wells.



10. Incubate the cells at 37°C to target ideal confluency of 50–70% in each well. Do not allow cell overgrowth.

    As an example, Element incubates HeLa cells for 16 to 18 hours.

Fix Cultured Adherent Cells

1. Gather the following consumables:
   - » 1X Dulbecco's Phosphate Buffered Saline (DPBS), pH 7–7.4, sterilized
   - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
   - » Formaldehyde, 4% (Fixation reagent)
   - » If storing the slide, 40 U/µl RiboLock RNase inhibitor diluted to 0.1 U/µl with 1X PBS
2. Warm the 1X DPBS in a 37°C water bath.
3. To remove the cell culture medium, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.

   > Do not fix more than three slides at the same time to avoid cells from drying out during the process.

4. Carefully wash each well with the appropriate volume of 1X DPBS to remove dead cells. Slightly tip the slide kit and slowly dispense along the middle of each well wall.
   - » 12-well slide—200 µl
   - » 1-well slide—3 ml
5. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
6. Repeat the wash one more time.
7. Slightly tip the slide kit and slowly add the appropriate volume of fixation reagent along the middle of each well wall.
   - » 12-well slide—150 µl
   - » 1-well slide—2 ml
8. With a lid on the slide kit, incubate at room temperature for 20–30 minutes.

   Fixation time varies by cell line. Do not exceed 30 minutes.
9. To remove the fixation reagent, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
10. Carefully wash each well with the appropriate volume of *1X PBS*. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.
    - » 12-well slide—200 µl
    - » 1-well slide—3 ml
11. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
12. Repeat the wash two more times. Do not remove the liquid after the final wash.
13. After fixing cells, proceed to one of the following options:
    - » Perform a cytoprofiling run on the AVITI24 System. See *Run Preparation and Setup* on page 18.
    - » Assess success of sample preparation. See *Teton Optimization Kit* on page 37.
    - » If you plan to ship samples, see *Shipping Samples* on page 47.
14. If not proceeding immediately, do the following:
    a. Remove liquid from the final wash.
    b. Add the appropriate volume of 0.1 U/µl RiboLock RNase inhibitor to each well, ensuring the surface of each well is covered.
       - ◦ 12-well slide—60 µl
       - ◦ 1-well slide—1 ml
    c. Cover the wells with an adhesive seal and store samples at 2°C to 8°C for up to 30 days.

Attach and Fix Suspension Cells

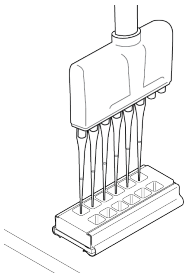
Sample preparation of suspension cells involves steps to attach cells to the Teton slide kit and then fix attached cells.

- **Attach**—The step to attach and immobilize live suspension cells to the treated slide surface using centrifugation.
- **Fix**—The fixation step crosslinks cells to the slide while halting cell function and preserving the structure of the bound cells.

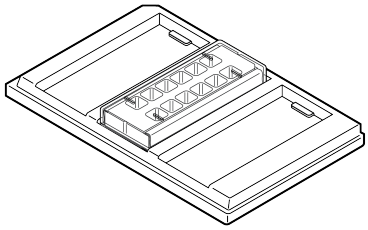
Attach Suspension Cells

1. Gather the following consumables:
   - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
   - » Teton Slide Kit
   - » 15 ml Falcon tube
   - » Assembly holder, quantity 2
     (ProPlate Tray, Grace Bio-Labs, part # 246879)
2. To ensure PBS remains sterile, always open the PBS bottle inside the biosafety cabinet.
3. Centrifuge the cells for 5 minutes at 300 x g in a 15 ml or 50 ml Falcon tube depending on final volume.
4. Use a pipette to remove the supernatant without disturbing the cell pellet.
5. Add 5 ml 1X PBS to resuspend the cell pellet and dilute the cell solution depending on desired confluency.

   If using Jurkat cells, for example, dilute to 400–600 K cells/ml. Optimize depending on cell size or final application.
6. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently load the appropriate volume of cell solution in the center of the well. Do not swirl the pipette when loading. Do not disturb the surface.
   - » 12-well slide—150 µl
   - » 1-well slide—1.5 ml



7. Cover the wells with the slide kit lid.
8. Load the covered slide kit onto an assembly holder.



9. Balance the centrifuge with another assembly holder. If preparing more than one slide kit, divide the slide kits between the two assembly holders.
10. Centrifuge at 300 x g for 15 minutes.

Fix Attached Suspension Cells

1. Gather the following consumables:
   - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
   - » 8% Formaldehyde (Fixation reagent)
   - » (Optional) 40 U/µl RiboLock RNase inhibitor diluted to 0.1 U/µl with 1X PBS
2. Remove the assembly holder from the centrifuge, remove the slide kit from the holder, and remove the lid.
3. Do not remove any liquid from the wells.
4. Slightly tip the slide kit and slowly add the appropriate volume of fixation reagent (8% formaldehyde) along the middle of each well wall. ***Do not pipette to mix.***
   - » 12-well slide—150 µl
   - » 1-well slide—1.5 ml
5. Cover the wells with the slide kit lid and incubate at room temperature for 20–30 minutes.

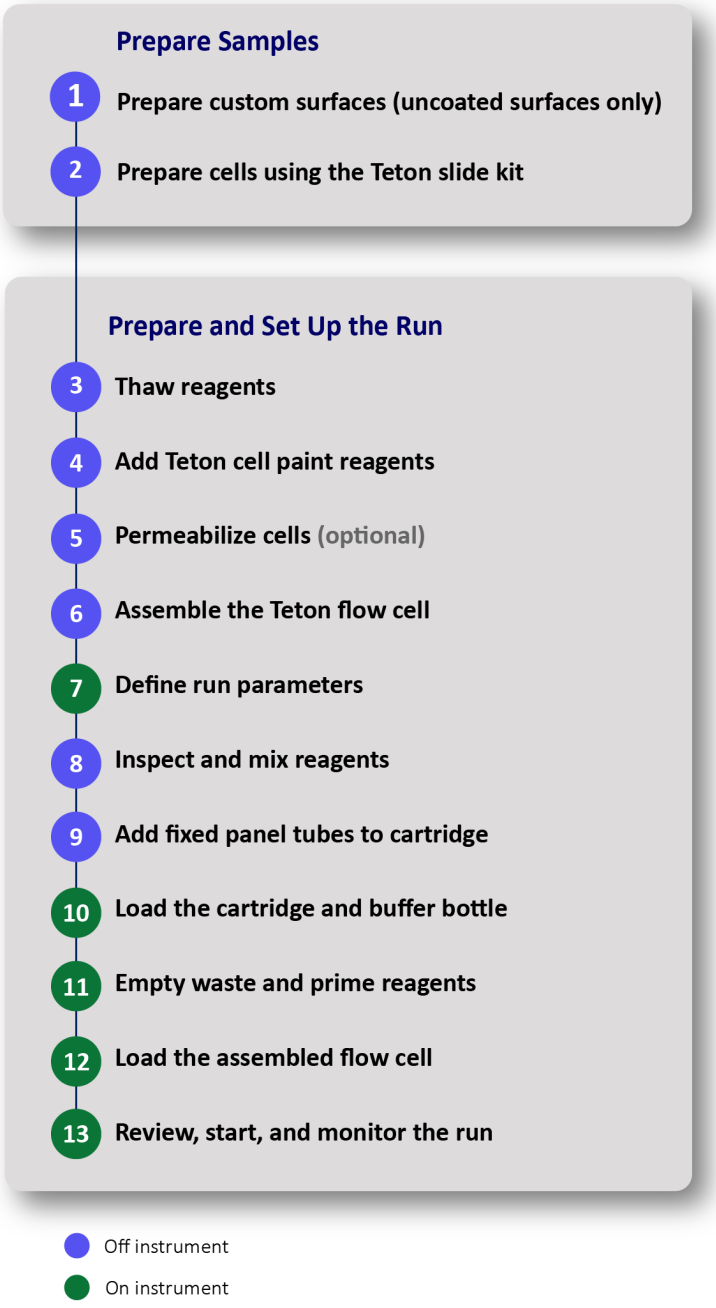
   Fixation time varies by cell line. Do not exceed 30 minutes.
6. Carefully wash each well with the appropriate volume of *1X PBS*. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.
   - » 12-well slide—200 µl
   - » 1-well slide—3 ml
7. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
8. Repeat the wash two more times. Do not remove the liquid after the final wash.
9. After fixing cells, proceed to one of the following options:
   - » Perform a cytoprofiling run on the AVITI24 System. See *Run Preparation and Setup* on page 18.
   - » Assess success of sample preparation. See *Teton Optimization Kit* on page 37.
   - » If you plan to ship samples, see *Shipping Samples* on page 47.
10. If not proceeding immediately, do the following:
    a. Remove liquid from the final wash.
    b. Add the appropriate volume of 0.1 U/µl RiboLock RNase inhibitor to each well, ensuring the surface of each well is covered.
       - ◦ 12-well slide—60 µl
       - ◦ 1-well slide—1 ml
    c. Cover the wells with an adhesive seal and store samples at 2°C to 8°C for up to 30 days.

CHAPTER 4

Run Preparation and Setup

Performing a cytoprofiling run on an AVITI24 System includes steps to prepare reagents and cell samples, assemble the flow cell, and then follow prompts on the AVITI OS interface to setup the run.

Teton Run Preparation Summary



Prepare Reagents

Preparing reagents for a cytoprofiling run requires thawing the reagent cartridge, cell paint reagents, and fixed panel tubes.

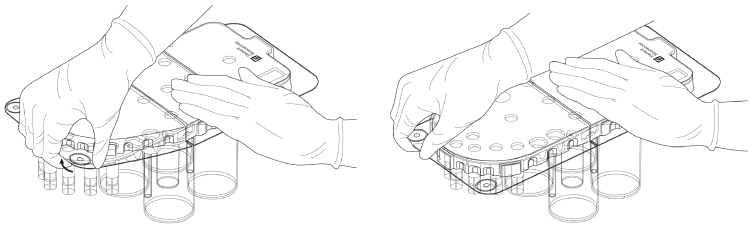
Thaw Reagent Cartridge

1. Remove a cartridge from -25°C to -15°C storage.

   CAUTION
   The cartridge contains light-sensitive reagents. ***Protect the cartridge from light***.

2. Remove the shipping cover:

   a. While supporting the cartridge, lift the removal tab at the left corner until it releases from the cartridge.



   b. Moving across the front edge of the shipping cover, repeatedly lift the edge until the cover is fully released.

   c. Pull to remove the remainder of the shipping cover from the cartridge.

3. Place the cartridge in a room-temperature water bath and thaw for ~3 hours. Do not submerge.
4. Inspect each well to make sure all reagents are fully thawed. Reagents thaw at varying rates.

   If ice remains in any well, return the cartridge to the water bath until fully thawed.

5. Set aside the thawed cartridge at room temperature. If not immediately initiating the run, place the thawed cartridge at 2°C to 8°C. Do not exceed 3 hours.

Thaw Cell Paint Reagents

1. When the reagent cartridge is almost thawed, remove Teton Reagent A and Teton Reagent B from -25°C to -15°C storage.
2. Thaw reagents in a room temperature water bath for 20 minutes.

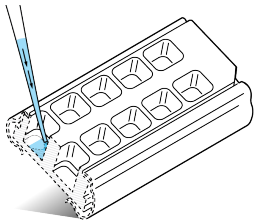
Thaw Fixed Panel Tubes

If you are using a Teton cartridge and a fixed panel, thaw the protein and RNA tubes before use.

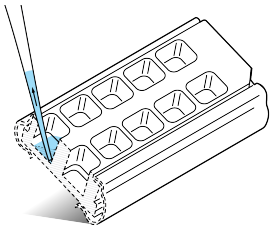
1. Check the expiration date on the fixed panel box before proceeding.
2. When the reagent cartridge is almost thawed, remove protein tube and RNA tube provided in the fixed panel kit from -25°C to -15°C storage.
3. Thaw reagents in a room temperature water bath for 15 minutes.
4. If you are using the Teton Custom Add-On Protein Panel Assembly Kit, see *Teton Custom Add-On Protein Panel* on page 34.

Add Cell Paint Reagents A and B

1. Ensure Teton Reagent A and Teton Reagent B are thawed.
2. Invert each tube 10 times to mix and then briefly centrifuge. *Do not vortex*.
3. Remove samples from 2°C to 8°C storage.
4. To remove the liquid from the slide kit, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
5. Wash each well with the appropriate volume of 1X PBS. Slightly tip the slide kit and slowly add liquid along the middle of each well wall. Do not pipette up and down.
    - 12-well slide kit—200 µl
    - 1-well slide kit—2 ml
6. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
7. Slightly tip the slide kit and slowly add the appropriate volume of Teton Reagent A along the middle of each well wall.
    - » 12-well slide—80 µl
    - » 1-well slide—1.5 ml



8. Incubate at room temperature for 10 minutes.
9. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
10. Wash each well with the appropriate volume of 1X PBS. Slightly tip the slide kit and slowly add liquid along the middle of the wall of each well.
    - » 12-well slide—200 µl
    - » 1-well slide—2 ml
11. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.



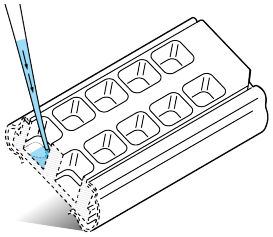
12. Repeat the wash two more times.
13. Slightly tip the slide kit and slowly add the appropriate volume of Teton Reagent B along the middle of the wall of each well.
    - » 12-well slide—80 µl
    - » 1-well slide—1.5 ml
14. Incubate at room temperature for 10 minutes.
15. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.

16. Wash each well with the appropriate volume of 1X PBS. Slightly tip the slide kit and slowly add liquid along the middle of the wall of each well.
    - » 12-well slide—200 µl
    - » 1-well slide—2 ml
17. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
18. Repeat the wash two more times. Do not remove the liquid after the final wash.
19. Proceed to one of the following steps:
    - » *Permeabilize Cells* (optional)
    - » *Assemble the Teton Flow Cell*. Leave the cells in 1X PBS until you assemble the flow cell.

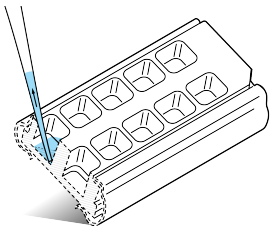
Permeabilize Cells

The Element protocol *does not require* permeabilization. If you permeabilize the samples, use ethanol and do not exceed a 10-minute incubation. Minimize the time between permeabilization and starting the run.

1. Prepare permeabilization reagent (70% EtOH)—Prepare fresh daily.
    - » 7 ml ethanol, biological grade
    - » 3 ml biological-grade/RNase-free water
2. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
3. Slightly tip the slide kit and slowly add the appropriate volume of permeabilization reagent along the middle of each well wall.
    - » 12-well slide—150 µl
    - » 1-well slide—1.5 ml



4. Cover the slide kit and incubate for 1–10 minutes at room temperature.
5. To wash the wells, slightly tip the slide kit and remove the appropriate volume of permeabilization reagent from each well. Then, slowly add the appropriate volume of 1X PBS along the middle of each well wall.
    - » 12-well slide—100 µl
    - » 1-well slide—1 ml



6. Repeat the wash three more times. Do not remove the liquid after the final wash.
7. Proceed immediately to *Assemble the Teton Flow Cell*.

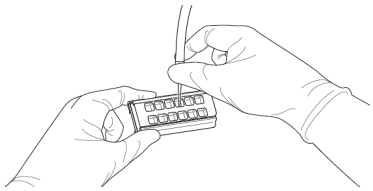
Assemble the Teton Flow Cell

Assembling the Teton flow cell requires the following parts and recommended equipment:

- Teton flow cell aligner
- Teton flow cell sealer
- Teton flow cell assembly kit
- Vacuum aspiration system with 200 µl tip (recommended)

Disassemble the Slide Kit

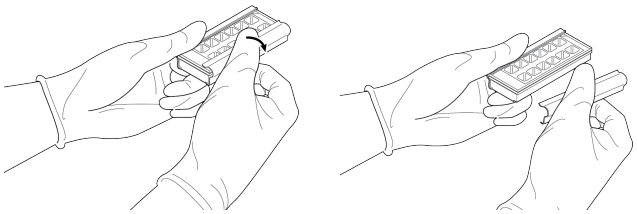
1. Use a vacuum aspiration system with a 200 µl tip to remove the liquid from each well of the slide kit:
   a. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface.
   b. Make sure no flowing liquid is observed.



2. Turn the slide kit upside down so the open wells are facing downward and the glass slide is facing upward.

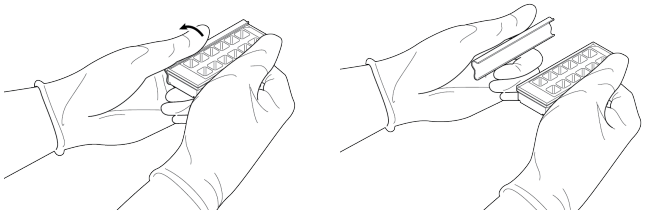
   CAUTION
   Disassemble the slide kit as described to avoid breakage or damage to the edges.

3. Holding the slide kit with both hands on the long edges, place your thumb on the top-center location of one of the side clips. With smooth and consistent movement, rotate the top edge of the side clip outward to release the clip.



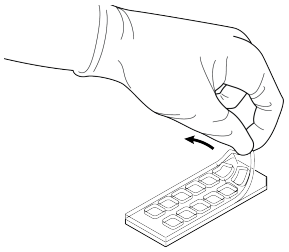
> ***Do not pull the side clip from the end regions of the clip.*** Always remove the clips from the center of each clip to avoid damage to the slide.
> ***Do not apply pressure on the slide surface.*** Always hold the slide kit from the edges to avoid damage to the slide.

4. To release the second clip, place your thumb on the top-center location of the side clip, and rotate the top edge of the side clip outward with smooth and consistent movement.



5. Lift the top-right beveled corner of the gasket to allow some air between the gasket and the frame. Then, firmly lift the frame from the gasket.

6. Grip the top-right corner of the slide kit gasket and gently pull to remove it from the sample slide.



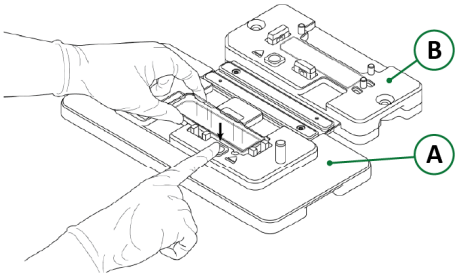
Align and Seal the Slides

1. Make sure the surface of the Teton flow cell aligner is clean. Thoroughly wipe both sides of the aligner, including all pins.

   CAUTION
   Handle slides with care to avoid breakage or damage to the edges. Take care when aligning and sealing slides.

2. Load the sample slide onto the Teton flow cell aligner:

   a. Press and hold the button on the **Sample** side of the flow cell aligner.
   b. Align the beveled corner of the sample slide with the beveled corner markings on the aligner.
   c. Make sure the sample slide is well-seated in the recessed area, and release the button.



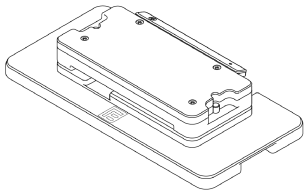
A **Sample** side
B **Adhesive** side

3. Open the flow cell assembly kit and remove the adhesive slide from the package. Handle the slide from the edges only and make sure the slide is free of debris.
4. Load the adhesive slide onto the flow cell aligner:

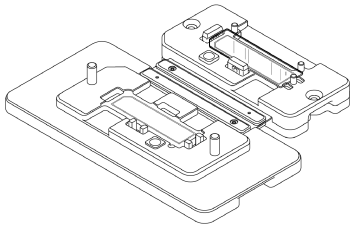
   a. Press and hold the button on the **Adhesive** side of the flow cell aligner.
   b. Align the beveled corner of the adhesive slide with the beveled corner markings on the aligner.
   c. Make sure the adhesive slide is well-seated in the recessed area, and release the button.

5. Starting from the beveled corner, peel off the protective easy-peel film from the adhesive slide.
6. Close the aligner to affix the sample slide and adhesive slide:

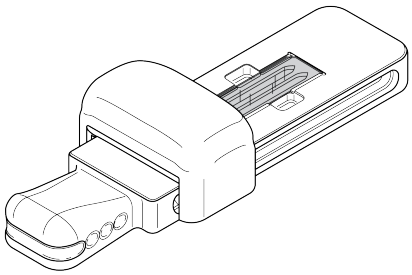
   a. Using two hands, one on each side, lift and fold the **Adhesive** side of the flow cell aligner over the **Sample** side.
   b. Align the posts on the **Sample** side with the holes on the Adhesive side.
   c. Guiding the Adhesive side with both hands, slowly allow the **Adhesive** side to make contact with the **Sample** side.
   d. Press gently for 5 seconds. Excessive pressure can damage the slides.



7. Lift the **Adhesive** side to open the flow cell aligner. The sample slide is affixed to the adhesive slide.



8. Make sure the surface of the Teton flow cell sealer is clean to avoid damage to the slide. Thoroughly wipe the recessed slide holder. For more information, see *Caring for the Teton Flow Cell Sealer* on page 46.
9. Place the aligned slides on the Teton flow cell sealer in the recessed slide holder. The slides must be well-seated to avoid damage to the slides.



10. Hold the flow cell sealer roller grip with one hand and the base handle with the other hand. *Very slowly* move the roller grip forward and then back, taking ~5 seconds to roll in each direction. Repeat the forward and back movement at least 3 times.

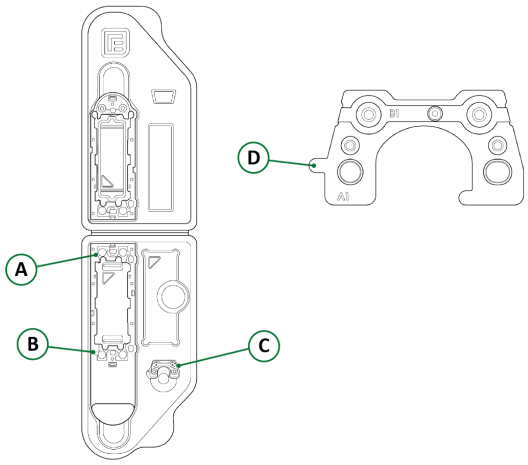
    NOTE
    Moving the roller grip slowly ensures a proper seal and avoids damage to the slides.

11. Flip over the aligned slides and reposition in the recessed slide area of the flow cell sealer. Repeat step 10 an additional 3 times.

Assemble the Flow Cell Cartridge

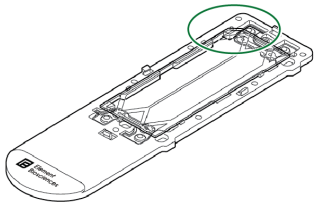
1. Position each of the two flow cell gaskets onto the bottom half of the flow cell cartridge, one above and one below the slide area. Make sure the gasket key is properly seated in the recess.

   CAUTION
   Gaskets ***must*** be present to prevent run failure.

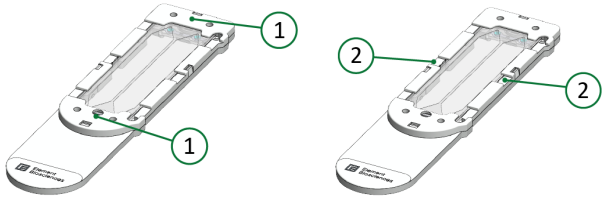


A Gasket location above slide area
B Gasket location below slide area
C Gaskets stored in flow cell assembly package
D Gasket key

2. Remove the slides from the flow cell sealer.
3. Place the sealed slides onto the bottom half of the flow cell cartridge with the beveled corner in the top-left position as shown on the packaging. The cartridge design ensures only one orientation. Make sure the slides rest flat on the cartridge bottom.



4. Align the top half of the flow cell cartridge over the bottom half.
5. Press down on the cartridge in four places to secure the top half to the bottom half until you hear a click.
   a. First, press down near the top and bottom of the slide area.
   b. Second, press down on each side of the slide area.



6. Visually inspect the flow cell cartridge to make sure there are no gaps along the sides of the cartridge top and bottom. If a gap is visible, repeat step 5 to ensure the cartridge top and bottom are fully engaged.
7. Wipe the assembled flow cell surface with an ethanol wipe and dry the surface with a lens wipe.
8. Proceed immediately to *Set Up a Cytoprofiling Run* on page 26.

Set Up a Cytoprofiling Run

1. If applicable, stage run manifests for import:
   - » If setting up the run manually, save the manifest on a USB and connect the USB drive to an instrument USB port.
   - » Alternatively, you can save the manifest to the specified SMB storage connection.
   - » If you planned the run in Elembio Cloud, upload the manifest to the planned run.
2. On the Home screen, select **New Run**.
3. For run type, select **Cytoprofiling**.
4. Select a side or both sides to use for the run.
   - » **Side A**—Set up a run on side A.
   - » **Both**—Set up simultaneous runs on sides A and B.
   - » **Side B**—Set up a run on side B.
5. Select **Next** and proceed to one of the following steps:
   - » For a **Manual Run**, proceed to *Define Manual Run Parameters*.
   - » For a **Planned Run**, proceed to *Select a Planned Run*.

Define Manual Run Parameters

1. Make sure **Manual Run** is selected for the type of run.
2. In the Run Name field, enter a unique name to identify the run.

   The field accepts 1–64 alphanumeric characters, hyphens (-), and underscores (_).
3. [Optional] In the Run Manifest field, select **Browse** and import a run manifest.

   You can import a run manifest from an inserted USB drive or from an SMB storage connection.
4. [Optional] In the Description field, enter a description that represents the run.

   The field accepts ≤ 500 alphanumeric characters, hyphens, underscores, spaces, and periods (.).
5. In the Storage drop-down menu, select a storage location or leave the default selection.
6. In the Well Layout field, select 12 wells or 1 well.
7. In the Cartridge drop-down menu, select the cartridge type you are using.
8. If you selected Teton cartridge, select the fixed panel you are using from the Fixed Panel drop-down menu.

   NOTE
   Fixed panel kits are used with the Teton cartridge. Other cartridge types include fixed panel components.
9. If you are using a **Custom Add-On Protein Panel** prepared with the Teton Custom Add-On Protein Panel Assembly Kit, you can import the panel in two ways:
   - » If the panel is Published in Elembio Cloud, select the name of your panel from the drop-down menu.
   - » Otherwise, select **Upload a panel.json file** from an inserted USB drive.
10. In the Small Cell field, select **Yes** if you are using small cells. Otherwise, select **No**.

    Example cell lines that benefit from this setting include Jurkat, PBMC, MCF-7, PC-3, and HCT-116.
11. If you are using a custom recipe, select **Advanced Settings**. Select **Browse** and import the custom recipe file from a USB drive.
12. Select **Next** and repeat steps 2–12 to setup side B, or proceed to the Prepare Reagents screen.
13. Proceed to *Inspect and Mix Reagents* on page 27.

Select a Planned Run

1. Select **Planned Run**.

   AVITI OS displays a list of compatible planned runs for the instrument and run type. For information on planned run compatibility, see *Run Planning for Cytoprofiling* in the *Online Help*.
2. Select the run you want to use from the list of planned runs.
3. Review the run parameter fields to make sure they are correct.

   If you need to edit a planned run, modify it in Elembio Cloud. See *Edit a Planned Run* in the *Online Help*.
4. In the Storage drop-down menu, select the storage connection for the run.
5. Select **Next** to proceed to the Prepare Reagents or the Run Side B screen.
   - » After you proceed, the selected planned run becomes unavailable for other connected instruments.
   - » If you exit run setup before priming, the run returns to the list of available planned runs.
6. If applicable, repeat steps 2–5 to set up a dual start run with a second planned run.

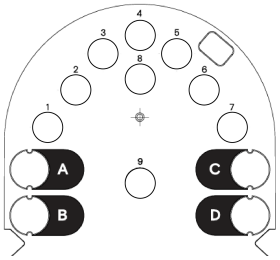
Inspect and Mix Reagents

1. Inspect each cartridge well to make sure reagents are fully thawed.
2. Gently invert the cartridge ***10 times*** to mix reagents.

   CAUTION
   Inadequately mixed reagents can cause run failure.
3. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.
4. Inspect the small tubes to make sure all liquid is at the bottom of the tube.
5. Place the cartridge into a clean cartridge basket and lock the clips. Wipe any excess moisture.

Add Fixed Panel Tubes to the Cartridge

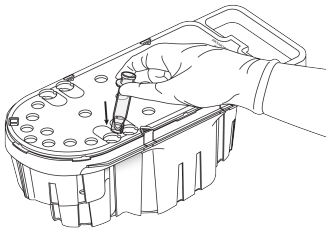
If you are using a Teton cartridge, add the thawed fixed panel tubes to the cartridge.

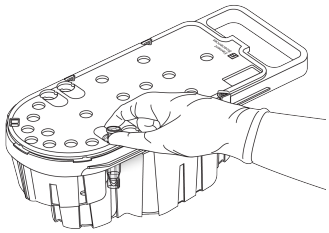


Position **A**—Protein tube
Position **B**—RNA tube

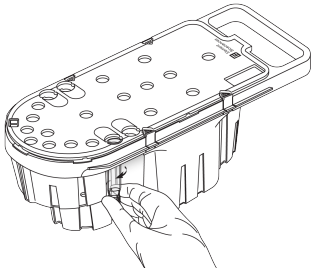
1. If the fixed panel protein tube is sealed, invert to mix the protein tube.

   Do *not* invert the tube if you are using a custom protein panel spike-in. The tube is not sealed.
2. From the top of the cartridge, insert the protein tube in position **A**.





3. Hold the bottom of the protein tube and turn clockwise 90° to lock the tube in position. Push upward on the tube to confirm a locked position.



4. Invert to mix the RNA tube.
5. From the top of the cartridge, insert the RNA tube in position **B**.
6. Hold the bottom of the RNA tube and turn clockwise 90° to lock the tube in position. Push upward on the tube to confirm a locked position.

Confirm Reagent Preparation

1. Select the **Invert cartridge** checkbox to confirm that reagents are mixed.
2. Select the **Insert into basket** checkbox to confirm that the cartridge is in the cartridge basket.
3. If you are using a Teton cartridge, confirm that the protein tube is loaded onto the cartridge.
   - » If using a fixed protein panel, select the **Invert and load protein tube** checkbox.
   - » If using a custom protein panel, select the **Verify protein tube is pierced and load tube** checkbox.
4. If you are using a Teton cartridge, select the **Invert and load RNA tube** checkbox to confirm that the RNA tube is loaded onto the cartridge.
5. Select the **Verify flow cell** checkbox to confirm that the flow cell assembly is complete with no gaps in the sides of the flow cell.
6. Select **Next** to proceed to the Load Reagents screen.

Load Cartridge and Buffer

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Slide the basket containing the thawed cartridge into the reagent bay until it stops.
4. Support the buffer bottle with both hands and slide it into the reagent bay until it stops.
5. Close the reagent bay door, and then select **Next** to proceed.

Empty Waste and Prime Reagents

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

   CAUTION
   Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.
4. Open the transport cap and the vent cap.
5. Support the waste bottle with both hands and empty the waste:

   a. Position the bottle over the funnel or waste receptacle.
   b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
   c. If necessary, wipe liquid off the bottle.
6. Close the vent cap and return the empty waste bottle to the waste bay.
7. Screw the transport cap onto the cap holder and close the waste bay door.

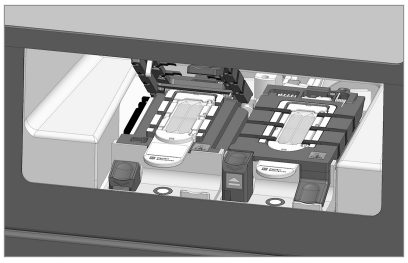
   NOTE
   Before priming, you can discard run setup and save the cartridge. Priming pierces reagent seals and prevents further use.
8. Select **Next** to ***automatically*** start priming. Priming takes approximately 24 minutes.
9. When priming is complete, select **Next** to proceed to the Load Flow Cell screen.

   AVITI OS moves the nest forward and opens the nest bay door. A brief delay is normal.

Load the Flow Cell

1. Make sure the nest status light is blue.
2. Press the button to the left of the nest to open the lid. Make sure to fully press down on the button.

   —Failure to fully press down on the button can cause errors when closing the lid or aligning the flow cell.—
3. Remove the used flow cell from the nest.
4. With the label facing up, place the assembled Teton flow cell over the three registration pins on the nest.



5. Lower the tab on the right side of the lid until the lid snaps into place.

   —The nest status light turns green.—
6. Select **Close Nest** to close the nest bay door and retract the stage.
7. Select **Next** to ***automatically*** start the Flow Cell Integrity Test.

   If the Flow Cell Integrity Test fails, you can recover the flow cell and save the run. See *Flow Cell Recovery* on page 32.
8. After the Flow Cell Integrity test successfully completes, select **Next**.

Review and Start the Run

1. On the Details page, review the run parameters:

Parameter	Description
Cartridge	The cartridge type
No. Wells	The number of wells on the flow cell
Panel	The fixed panel for the run
Storage	The location where run output is stored
Manifest	The file name of the uploaded run manifest, if applicable
Custom Add-On Protein Panel	If applied to the run, lists the name of the custom protein panel
Description	A description of the run (optional)
Advanced	If applicable, advanced run settings for the run, such a custom recipe

2. Select Consumable Information to review the flow cell, cartridge, and buffer bottle information:

Field	Description
Lot Number	The manufacturing batch number assigned to the consumable
Expires on	The date that the cartridge and buffer bottle expires
Serial Number	The unique identifier for the consumable or all zeros indicating an unscanned barcode
Part Number	The part identifier for the consumable

—A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.—

3. Select **Run** to start the run.
4. [Optional] If you imported run manifests from a USB drive, disconnect the USB drive.
5. Process the materials removed from the reagent bay:
   - » If you removed a used cartridge and buffer bottle, follow the instructions in *Discard the Cartridge and Bottle* on page 31.
   - » If you removed a wash tray, follow the guidelines for wash tray maintenance in the user guide for your instrument.

Monitor Run Metrics

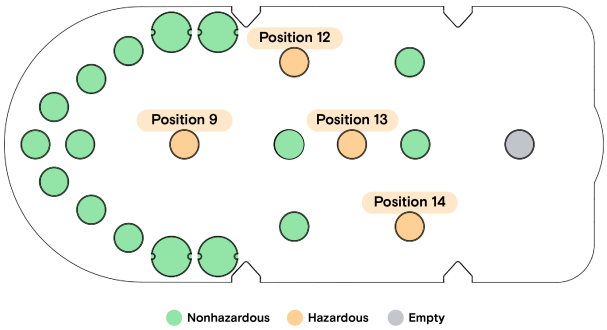
1. Select **Overview** or **Details** to toggle between views of run details.
2. Monitor run metrics as they appear onscreen. AVITI OS indicates the expected batch during which metrics appear.

   —Expected cycles are approximate, and all metrics are estimates.—
3. Continue monitoring the run as AVITI OS refreshes the metrics.
4. When the run is complete, leave all materials on the instrument.
   - » To return to the Details view, select **Overview**.
   - » To access run data, go to your storage location.

Discard the Cartridge and Bottle

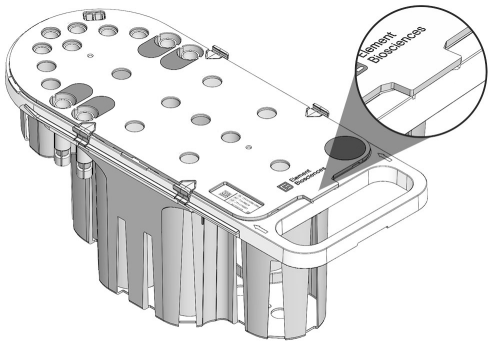
The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the Safety Data Sheets (SDS) at elementbiosciences.com/resources.

The following wells contain hazardous reagents. The position numbers in the figure align with the position numbers in the SDS.



Dispose of Reagents

1. Keep the cartridge in the basket with the clips locked.
2. Grip the lid tab and ***quickly and forcefully*** pull off the lid. Expect resistance.



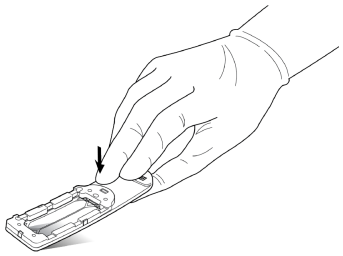
3. Remove the wells indicated as hazardous from the cartridge.
   —The volume remaining in each well depends on the number of cycles performed.—
4. Using a pipette tip or a similar tool, enlarge the hole in each foil seal to form a triangle.

5. Empty each well into hazardous waste or other appropriate container per the SDS.
6. Unlock the clips and remove the cartridge from the basket.
7. Remove the remaining wells from the cartridge and enlarge the hole in each foil seal.
8. Empty each well into the appropriate container per the SDS.
9. Discard the cartridge and buffer bottle per the SDS.
10. Rinse the basket with nuclease-free water and dry upside down.

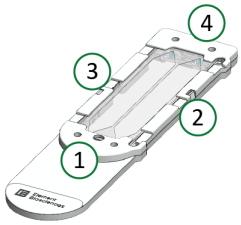
Flow Cell Recovery

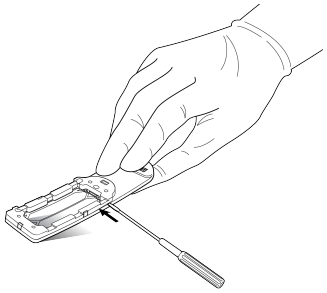
If the Flow Cell Integrity Test fails during run setup, perform the following steps to recover the flow cell.

1. Open the nest and remove the flow cell.
2. Use a vacuum aspiration system or pipette at the end ports to remove all liquid from the flow cell. Make sure the flow cell is completely dry.
3. Release each of the four snap positions that secure the top half of the cartridge to the bottom half in the order listed:
   a. With the flow cell on a flat surface, lift the cartridge handle upward with light pressure to slightly bend the cartridge and create a gap between the top half and the bottom half.

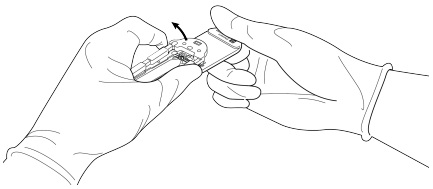


   b. Starting with position 1 below the slide area, insert a flat and rigid tool, such as a small screwdriver, into the gap along the side of the cartridge and toward the snap location. Maintain pressure on the cartridge and gently rotate the tool to release the snap.

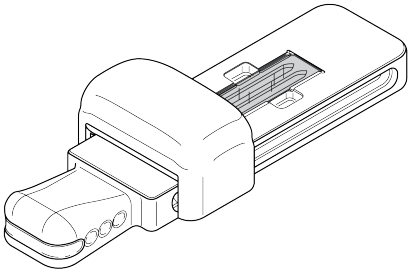




   c. With position 1 released, insert the tool at positions 2 and 3 along the side of the cartridge. Gently rotate the tool to release each snap.
   d. To release the snap at position 4, rotate the top half of the cartridge upward or use the tool from the side of the cartridge.



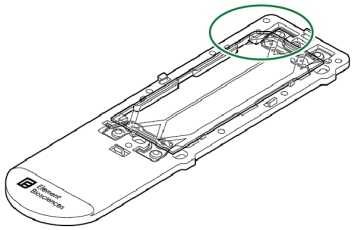
4. Lift to remove the slides from the bottom half of the flow cell cartridge.
5. Visually inspect the flow cell gaskets to make sure each gasket is well-seated.
6. Re-seal the slides using the flow cell sealer:
   a. Make sure the surface of the Teton flow cell sealer is clean. Thoroughly wipe the recessed slide holder.
   b. Place the affixed slides in the recessed slide holder. The slides must be well-seated to avoid damage to the slides.



c. Hold the flow cell sealer roller grip with one hand and the base handle with the other hand. *Very slowly* move the roller grip forward and then back, taking ~5 seconds for each pass. Repeat at least 3 times.

d. Flip over the slides and reposition in the recessed slide area of the flow cell sealer. Repeat the forward and back roller motion an additional 3 times.

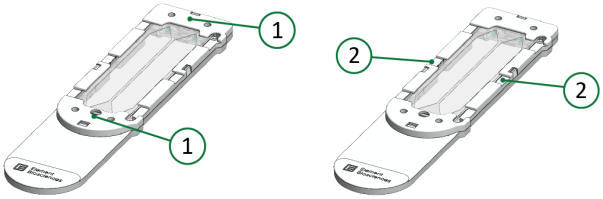
7. Place the sealed slides onto the bottom half of the flow cell cartridge with the beveled corner in the top-left position. Make sure the slides rest flat on the cartridge bottom.



8. Align the top half of the flow cell cartridge over the bottom half.
9. Press down on the cartridge in four places to secure the top half to the bottom half until you hear a click.

a. First, press down near the top and bottom of the slide area.

b. Second, press down on each side of the slide area.



10. Visually inspect the flow cell cartridge:

» Make sure there is no damage to the plastic and that the flow cell cartridge rests flat on a flat surface.

» Make sure there are no gaps along the sides of the cartridge top and bottom.

11. Wipe the assembled flow cell surface with an ethanol wipe and dry the surface with a lens wipe.
12. Reload the flow cell on the instrument and make sure the flow cell is well-seated on the nest.

» If the flow cell is well-seated on the nest, follow the software prompts to resume the run.

» If the flow cell does not seat properly on the nest or the Flow Cell Integrity Test fails again, you must cancel the run.

CHAPTER 5

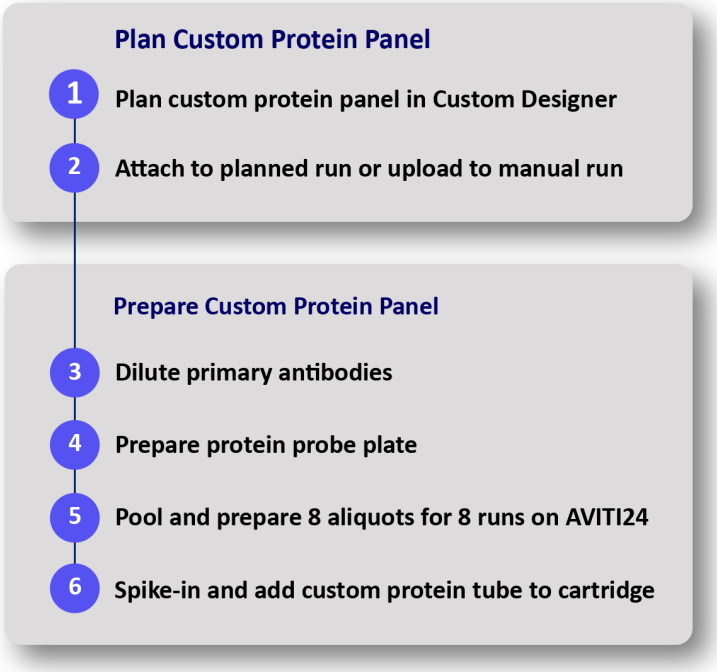
Teton Custom Add-On Protein Panel

The Teton Custom Add-On Protein Panel Assembly Kit enables an additional 88 protein targets of your choosing to customize a cytoprofiling run with targets of your interest. Your selection of up to 88 targets are pooled with the 50 protein targets in a Teton fixed panel kit. This add-on option is compatible with Teton cartridges only, part # 820-00036.

The Teton Custom Add-On Protein Panel Assembly Kit includes a reagent plate with different Teton detection probes in each well to combine with your selected protein targets. One kit supports eight Teton runs on the AVITI24 System.

To plan your custom protein panel, use the interactive Custom Designer tool through Elembio Cloud. Your planned custom protein panel can be attached to a planned run or imported for a manual run from the cloud or a USB drive as a panel.json file. Custom protein panels of 24-plex or fewer require a Teton Diversity Spike-In.

Teton Custom Add-On Protein Panel Protocol



Prerequisites and Planning

1. Confirm that primary antibodies are compatible.

   NOTE
   Only rabbit antibodies are compatible with this protocol.

2. Use Custom Designer to plan your custom protein panel and plate layout.

   a. Log in to your Elembio Cloud account and navigate to the Custom Designer section.

   b. Follow the onscreen instructions to create a new custom protein panel design.

3. Determine plexity. If your custom protein panel design is 24-plex or fewer, you must use a Teton Diversity Spike-In with this protocol.

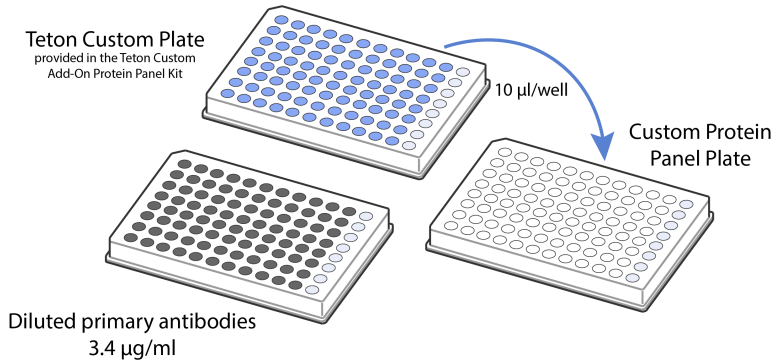
Thaw Consumables and Dilute Primary Antibodies

1. Gather the following consumables from the Teton Custom Add-On Protein Panel Assembly Kit from -25°C to -15°C storage:
   - » Teton Custom Add-On Protein Buffer
   - » Teton Custom Plate provided in the Teton Custom Add-On Protein Panel Kit (do not remove from pouch)
   - » Teton Custom Add-On Protein Control
2. Thaw the following kit components at room temperature for 15 minutes and then place on ice until use.
   - » Teton Custom Add-On Protein Buffer
   - » Teton Custom Plate (do not remove from pouch)
   - » Teton Custom Add-On Protein Control
3. Use the Teton Custom Add-On Protein Buffer to dilute each primary antibody to 3.4 µg/ml (22 nM) using one of the following methods:
   - » Dilute directly in each well of your 96-well primary antibody plate.
   - » Dilute in tubes and then transfer the diluted primary antibodies to a 96-well plate.

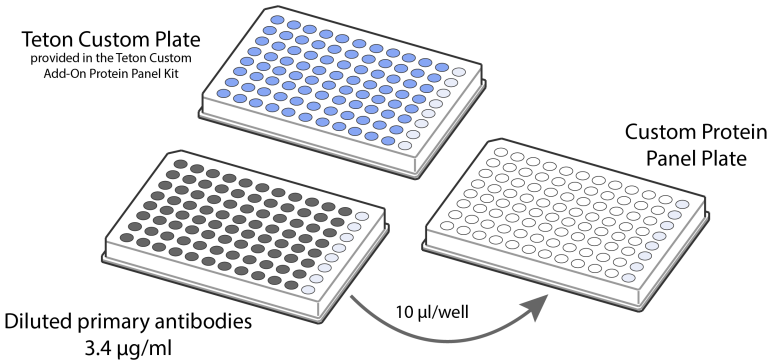
Prepare the Custom Protein Panel Plate

1. When thawed, remove the Teton Custom Plate from the pouch and briefly centrifuge.
2. Remove the foil seal and pipette to mix each well.
3. Label a new 96-well plate to indicate that this plate is the **custom protein panel plate**.
4. Transfer 10 µl from each well of the Teton Custom Plate to the corresponding well of the custom protein panel plate.

   For example, transfer from wells A1–H1 to wells A1–H1 using a multichannel pipette.



5. Transfer 10 µl from each well of your diluted primary antibody plate to the corresponding well of the custom protein panel plate. Pipette to mix after each transfer.



6. Seal the custom protein panel plate and briefly centrifuge to remove bubbles.
7. Incubate at room temperature for 1 hour.

Pool and Aliquot Proteins

1. Add 18 µl from each well of the custom protein panel plate to a 2 ml tube.

   For an 88-plex plate, expect a pooled volume of 1584 µl.
2. Add 36 µl of the Teton Custom Add-On Protein Control to the pooled panel.

   For an 88-plex plate, expect a pooled volume of 1620 µl.
3. If your custom panel is fewer than 88-plex, add the appropriate volume of Teton Custom Add-On Protein Buffer to the pooled panel to result in a volume of 1620 µl using the following formula:

   1584 – (Plexity x 18) = Buffer volume

   For example, if your panel is 64-plex, add 432 µl Teton Custom Add-On Protein Buffer.
4. Using eight low-bind tubes, transfer 200 µl pooled protein panel to each tube.

   NOTE
   Errors in pipetting can result in fewer than eight aliquots.
5. Label each tube with panel plexity and sample information.
6. Store unused aliquots at -25°C to -15°C for up to 30 days.

Spike-In Custom Panel to Protein Tube

Complete the following steps during run setup after thawing the cartridge. See *Run Preparation and Setup* on page 18.

1. If your custom panel is 24-plex or fewer, thaw the Teton Diversity Spike-In at room temperature for 15 minutes.
2. After the Teton cartridge is fully thawed, invert the cartridge 10 times to mix and insert it into a cartridge basket.
3. Make sure the fixed panel protein tube is thawed. Invert the tube several times to mix.
4. Using a clean pipette tip, pierce the foil seal of the fixed panel protein tube.
5. From one of the prepared 200 µl aliquots, add 180 µl pooled protein panel to the fixed panel protein tube. Pipette to mix.
6. If your custom panel is 24-plex or fewer, add 11 µl Teton Diversity Spike-In to the fixed panel protein tube. Pipette to mix.

   The protein tube with your custom protein panel is ready to load onto the Teton cartridge.
7. Proceed to *Add Fixed Panel Tubes to the Cartridge* on page 27.

CHAPTER 6

Teton Optimization Kit

Use the Teton Optimization Kit to assess sample quality, cell growth, and morphology after sample preparation. The kit provides reagents to prepare a 12-well slide kit containing prepared cells for viewing under a microscope.

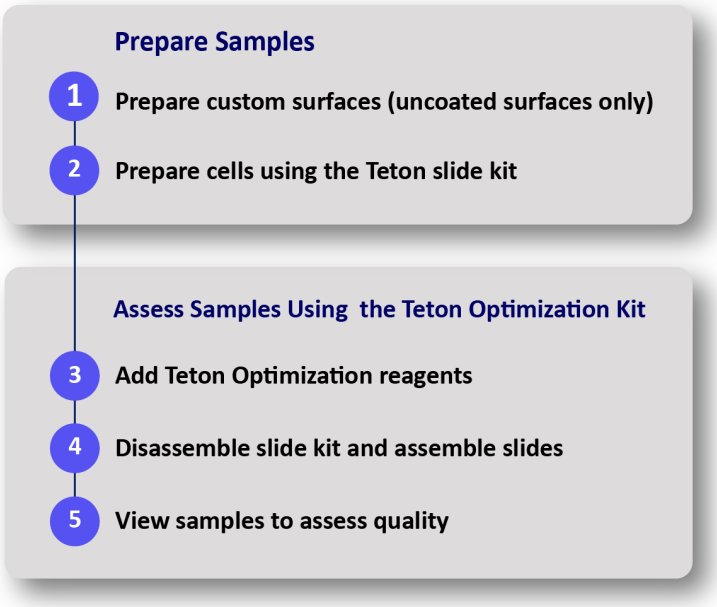
Requirements

- Prepared cell samples on a 12-well slide kit (see *Sample Preparation* on page 13)
- Slide holder adapter, Agilent BioTek, catalog # 1220548

Microscope Specifications

Item	Specification
Microscope	Fluorescent
Filters	Green: 515–560 nm excitation, 580–650 nm emission (similar to Cy3 filter for cell membrane) Red: 620–650 nm excitation, 660–750 nm emission (similar to Cy5 filter for cell nucleus)
Objective	Any fluorescence objective that can image through 1 mm thick glass slide, such as Olympus UCPLFLN20X Higher NA objectives achieve better resolution and fluorescent signal

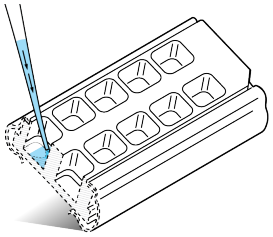
Teton Optimization Kit Protocol



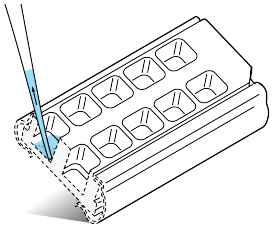
Add Teton Optimization Reagents

Perform all steps in a biosafety cabinet.

1. Remove the following components provided in the Teton Optimization Reagent Kit from -25°C to -15°C storage:
   - » Teton Optimization Reagent
   - » Teton Optimization Wash Buffer
2. Thaw reagents in a room temperature water bath for 15–20 minutes.
3. Invert each tube 10 times to mix.
4. If samples were stored after the fixation step, remove samples from 2°C to 8°C storage.
5. To remove the liquid from the slide kit, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
6. Wash each well with 150 µl 1X PBS. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.



7. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.

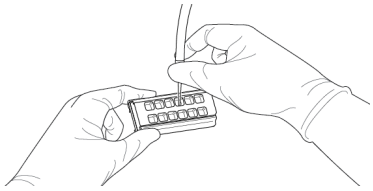


8. Repeat the wash one more time. Pipette to dry each well before proceeding. Make sure no flowing liquid is observed.
9. Add 70 µl of Teton Optimization Reagent to each well. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.
10. Incubate at room temperature for 1 minute. Do not exceed 2 minutes.

    CAUTION
    Over-incubation can lead to saturation of signal in certain cell lines.
11. Remove the Teton Optimization Reagent from each well. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface. Make sure all liquid is removed.
12. Add 150 µl of Teton Optimization Wash Buffer to each well. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.
13. Remove the Teton Optimization Wash Buffer from each well. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface.
14. Repeat the wash two more times.

Disassemble the Slide Kit

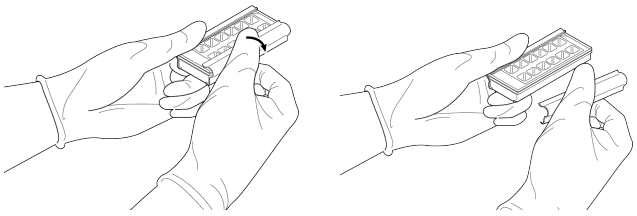
1. Use a vacuum aspiration system with a 200 µl tip to remove the liquid from each well of the slide kit:
   a. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface.
   b. Make sure no flowing liquid is observed.



2. Turn the slide kit upside down so the open wells are facing downward and the glass slide is facing upward.

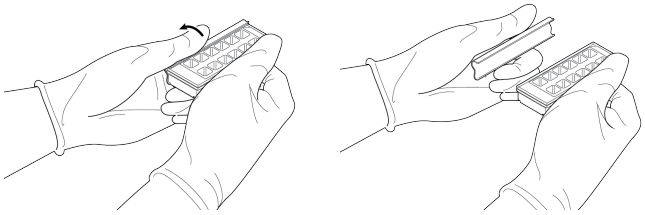
CAUTION
Disassemble the slide kit as described to avoid breakage or damage to the edges.

3. Holding the slide kit with both hands on the long edges, place your thumb on the top-center location of one of the side clips. With smooth and consistent movement, rotate the top edge of the side clip outward to release the clip.

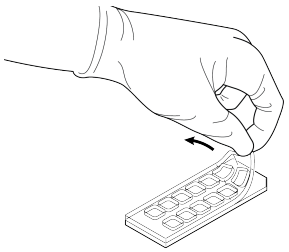


> ***Do not pull the side clip from the end regions of the clip.*** Always remove the clips from the center of each clip to avoid damage to the slide.
> ***Do not apply pressure on the slide surface.*** Always hold the slide kit from the edges to avoid damage to the slide.

4. To release the second clip, place your thumb on the top-center location of the side clip, and rotate the top edge of the side clip outward with smooth and consistent movement.



5. Lift the top-right beveled corner of the gasket to allow some air between the gasket and the frame. Then, firmly lift the frame from the gasket.
6. Grip the top-right corner of the slide kit gasket and gently pull to remove it from the sample slide.

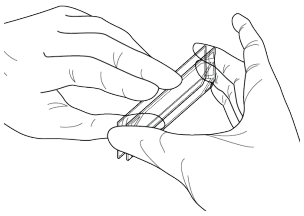


Manually Assemble Slides

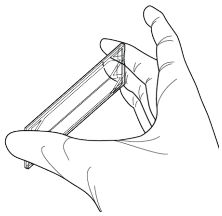
To manually assemble slides by hand, you must carefully align the slides before allowing the adhesive slide to touch the sample slide. Misalignment can prevent the slides from fitting into the slide holder adapter.

> Manual slide assembly is acceptable *only* when using the optimization kit for microscope viewing.
> If you have a flow cell aligner, use the flow cell aligner to assemble the slides. See *Align and Seal the Slides* on page 23.

1. Remove the adhesive slide from the Teton flow cell assembly kit.
2. Starting from the beveled corner, peel off the protective film from the adhesive slide.
3. Hold the sample slide with one hand and the adhesive slide with the other hand. Make sure the beveled corners are aligned.
4. Using your fingers on the edges of each slide, align the two slides but do not allow them to touch.



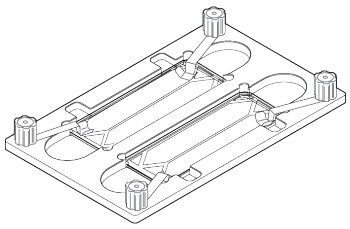
5. Working slowly to maintain alignment, allow the slides to come together.



6. Press to securely adhere the slides and remove any air.

Load Slides onto Microscope

1. Load slides onto a slide holder adapter.



For an inverted microscope:
- Place the beveled corner of the slides at the top-right side of the holder.
- In this position, cells are on the bottom surface for imaging.

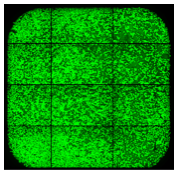
For an upright microscope:
- Place the beveled corner of the slides at the top-left side of the holder.
- In this position, cells are on the top surface for imaging.

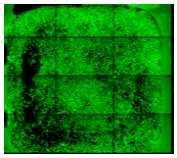
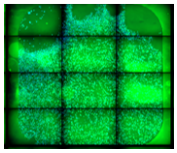
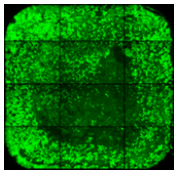
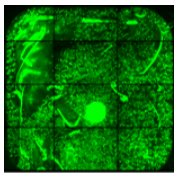
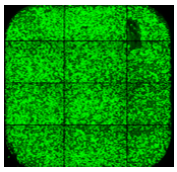
2. To adjust autofocus, consider a 1 mm thickness of the sample slide.
3. Collect 3–4 Z stack images with 2 µm spacing for optimal spatial resolution.

   For cells with a greater Z variance, increase stacks as necessary.

Troubleshoot Sample Preparation

The following images show results of successful and unsuccessful sample preparation.

Successful Result		Recommendation
Successful preparation technique		To ensure best results, practice pipetting techniques, such as slow pipetting speed. Avoid creating bubbles or scratching the surface. Follow protocol guidelines for slide kit and pipette position as described in *Protocol Guidelines* on page 13.

Unsuccessful Result		Recommendation
Ring pattern		Avoid circular movement of the pipette or the slide kit when dispensing cells. Use a side-to-side movement for a uniform cell distribution.
Cell loss in the corners of the well		Pipette slowly to prevent bubbles when dispensing cells. Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.
Cell loss in the middle of the well		Pipette slowly to reduce liquid impact on the slide surface after cell attachment.
Scratch marks on the surface		Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.
Cell loss in random locations		Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.

CHAPTER 7

Consumables and Tools

This section lists available Teton kits and tools for use with the AVITI24 System and user-supplied consumables. Promptly store the components at the specified temperatures upon receipt. For Safety Data Sheet (SDS) information, see elementbiosciences.com/resources.

Teton Cartridge and Reagent Kits

Teton Cartridge and Reagent Kit – 12 Well, # 860-00038

Part #	Component	Quantity	Shipping	Storage
820-00002	AVITI Buffer Bottle (Universal Wash Buffer)	1	Room temperature	Room temperature
820-00036	Teton Cartridge	1	-25°C to -15°C	-25°C to -15°C
830-00027	Teton Reagent Kit, 12 Well	1	-25°C to -15°C	-25°C to -15°C

Teton Cartridge and Reagent Kit – 1 Well, # 860-00037

Part #	Component	Quantity	Shipping	Storage
820-00002	AVITI Buffer Bottle (Universal Wash Buffer)	1	Room temperature	Room temperature
820-00036	Teton Cartridge	1	-25°C to -15°C	-25°C to -15°C
830-00033	Teton Reagent Kit, 1 Well	1	-25°C to -15°C	-25°C to -15°C

Teton Fixed Panel Kits

Teton Human Neuro Panel Kit, # 830-00038

Part #	Component	Quantity	Shipping	Storage
270-00229	Teton Neuro RNA Panel	1	-25°C to -15°C	-25°C to -15°C
270-00231	Teton Neuro Protein Panel	1	-25°C to -15°C	-25°C to -15°C

Teton Human Immuno Panel Kit, # 830-00039

Part #	Component	Quantity	Shipping	Storage
270-00230	Teton Immuno RNA Panel	1	-25°C to -15°C	-25°C to -15°C
270-00232	Teton Immuno Protein Panel	1	-25°C to -15°C	-25°C to -15°C

Teton Human MAPK-Cell Cycle Panel Kit, # 830-00040

Part #	Component	Quantity	Shipping	Storage
270-00206	Teton MAPK-CC RNA Panel	1	-25°C to -15°C	-25°C to -15°C
270-00208	Teton MAPK-CC-Apop Protein Panel	1	-25°C to -15°C	-25°C to -15°C

Teton Human MAPK-Apoptosis Panel Kit, # 830-00041

Part #	Component	Quantity	Shipping	Storage
270-00207	Teton MAPK-Apop RNA Panel	1	-25°C to -15°C	-25°C to -15°C
270-00208	Teton MAPK-CC-Apop Protein Panel	1	-25°C to -15°C	-25°C to -15°C

Teton Slide Kits

Teton Slide Kit, PLL – 12 Well (2-pack), # 860-00031

Part #	Component	Quantity	Shipping	Storage
810-00010	Teton Slide Kit, PLL - 12 Well	2	Room temperature	2°C to 8°C

Teton Slide Kit, PLL – 1 Well (2-pack), # 860-00029

Part #	Component	Quantity	Shipping	Storage
810-00009	Teton Slide Kit, PLL - 1 Well	2	Room temperature	2°C to 8°C

Teton Slide Kit, Uncoated – 12 Well (2-pack), # 860-00032

Part #	Component	Quantity	Shipping	Storage
810-00012	Teton Slide Kit, Uncoated - 12 Well	2	Room temperature	Room temperature

Teton Slide Kit, Uncoated – 1 Well (2-pack), # 860-00030

Part #	Component	Quantity	Shipping	Storage
810-00011	Teton Slide Kit, Uncoated - 1 Well	2	Room temperature	Room temperature

Teton Flow Cell Assembly Kits

Teton Flow Cell Assembly Kit, 12 Well (2-pack), # 860-00028

Part #	Component	Quantity	Shipping	Storage
810-00014	Teton Flow Cell Assembly Kit, 12 Well	2	Room temperature	Room temperature

Teton Flow Cell Assembly Kit, 1 Well (2-pack), # 860-00027

Part #	Component	Quantity	Shipping	Storage
810-00013	Teton Flow Cell Assembly Kit, 1 Well	2	Room temperature	Room temperature

Additional Teton Kits

Teton Optimization Kit, # 860-00022

Part #	Component	Quantity	Shipping	Storage
810-00014	Teton, Flow Cell Assembly Kit, 12 Well	2	-25°C to -15°C	-25°C to -15°C
830-00032	Teton Optimization Reagent Kit	2	-25°C to -15°C	-25°C to -15°C

Teton Custom Add-On Protein Panel Assembly Kit, # 860-00036

Part #	Component	Quantity	Shipping	Storage
830-00042	Teton Custom Add-On Protein Panel Kit	1	-25°C to -15°C	-25°C to -15°C
830-00044	Teton Custom Add-On Protein Buffer	1	-25°C to -15°C	-25°C to -15°C

Teton Diversity Spike-In, # 830-00043

Part #	Component	Quantity	Shipping	Storage
830-00043	Teton Diversity Spike-In (8 reactions)	1	-25°C to -15°C	-25°C to -15°C

Teton Flow Cell Assembly Tools

Teton Flow Cell Assembly Tool Set, # 860-00033

Part #	Component	Quantity
810-00016	Teton Flow Cell Aligner	1
810-00017	Teton Flow Cell Sealer	1

User-Supplied Consumables and Tools

Consumable	Supplier
Biological-grade/RNase-free water	General lab supplier
C-Chip cell counting chamber slides	InCyto, catalog # DHC-N01
Cell culture medium appropriate for cell line	General lab supplier
Compressed air duster	General supplier
Dulbecco's Phosphate Buffered Saline (DPBS), 1X, pH 7–7.4	Gibco, catalog # 14040117
Ethanol (EtOH), biological grade	General lab supplier
Ethanol wipes	General lab supplier
Formaldehyde	General lab supplier
Lens wipes	General lab supplier
Microseal 'B' adhesive seals, or equivalent	Bio-Rad, catalog # MSB1001
Phosphate-buffered saline (PBS), 1X, pH 7–7.4	General lab supplier
Pipette tips	General lab supplier
RiboLock RNase Inhibitor	ThermoFisher Scientific, catalog # EO0381
Water bath float	General lab supplier
For use with custom add-on protein protocol: 96-well plates 0.5 ml low-bind tubes 2 ml low-bind tubes	General lab supplier

Consumables for Surface Coatings

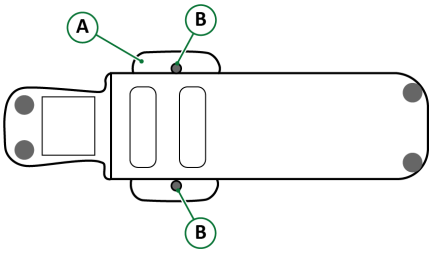
Surface Coating Type	Consumable	Supplier
All surface types	0.1 N NaOH solution Biological-grade/RNase-free water	General lab supplier
Collagen coating	Collagen Type 1, 4 mg/mL, stock solution Hydrochloric acid (HCl), 0.01 N	MilliporeSigma, C3867-1VL General lab supplier
Fibronectin coating	Fibronectin stock solution	MilliporeSigma, F1141-2MG
Gelatin coating	Gelatin solution, Type B, 2% in H_2O	MilliporeSigma, G1393-20ML
Laminin coating	Laminin stock solution (Laminin Mouse Protein, Natural)	Gibco, 23017-015
Matrigel coating	Matrigel stock solution (Matrigel Basement Membrane Matrix)	Corning, 356237
PLL coating	PLL stock solution, 0.01%	MilliporeSigma, P4707-50ML

Tools for Preparing Suspension Cells

Item	Supplier
Assembly holder (ProPlate Tray) Quantity 2 required for centrifuge balance	Grace Bio-Labs, part # 246879

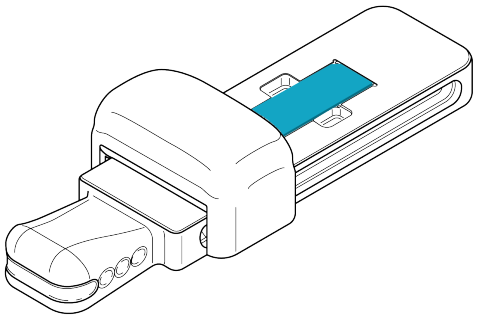
Caring for the Teton Flow Cell Sealer

- Ensure the entire top surface of the flow cell sealer is free of dust and debris before each use.
- To inspect the surface, make sure no slides are present, and then move the roller grip forward and backward. Use canned air to clean under the roller grip.
- If a slide has ever been damaged on the flow cell sealer, clean under the roller grip.
  - » From the bottom of the sealer, use a 3 mm hex head Allen key to loosen two captive screws and remove the roller grip cover.
  - » From the top of the sealer, use an ethanol wipe to clean the roller wheel.
  - » Tighten the two captive screws to reattach the roller grip cover.



A Roller grip
B Captive screws

- When using the sealer, always move the roller in a slow and deliberate movement. Moving the roller too quickly can damage the slide or slide edges.
- The flow cell sealer comes with a molded placeholder slide seated in the indented surface of the sealer. Store the sealer with the placeholder slide in place and the roller grip parked closest to the sealer handle.



Shipping Samples

To ship samples to another location after the fixation step, use the following instructions to prepare and package the slide kit.

1. Gather the following consumables:
   - » 1X Phosphate Buffered Saline (PBS)
   - » 40 U/µl RiboLock RNase inhibitor
   - » Adhesive seal, such as Microseal 'B'
2. If samples were stored after the fixation step, remove samples from 2°C to 8°C storage.
3. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
4. Add the appropriate volume of RiboLock RNase Inhibitor and 1X PBS to result in a 0.1 U/µl solution:
   - » 12-well slide—Add 6 µl RiboLock RNase Inhibitor to 2.4 ml 1X PBS.
   - » 1-well slide—Add 7.5 µl RiboLock RNase Inhibitor to 3 ml 1X PBS.
5. Add the appropriate volume of 1X PBS with RNase inhibitor to each well, ensuring at least 50% of the well volume.
   - » 12-well slide—200 µl
   - » 1-well slide—3 ml
6. Place an adhesive seal over the wells of the slide kit. Press firmly along the edges of the wells to secure the seal.
7. Do not reuse the slide kit lid. Instead, properly dispose of the lid as waste.
8. Store samples at 2°C to 8°C until ready to ship.
9. Prepare the slide kit for shipping:
   a. Add padding around the sealed slide kit.
   b. Place the slide kit in an empty pipette box, small freezer box, or similar.
   c. Place the box containing the slide kit in another box with cold packs.
   d. Seal and label the outer box with ↑ **This Side Up** to minimize impact to cells during shipping.
10. Ship samples according to local laws and regulations.

Document History

Revision	Description of Change
April 2025 Document # MA-00053 Rev. C	• Restructured guide with chapter headings to better organize workflow and add-on protocols. • Added fixed panel RNA and protein tubes to Prepare Reagents section. • Added instructions for adding fixed panel tubes to the cartridge during run setup. • Added Teton Custom Add-On Protein Panel Assembly Kit and Teton Diversity Spike-In and instructions for use. • Added Teton Cartridge and Reagent Kits and Teton fixed panel kits. • Added Universal Wash Buffer to AVITI Buffer Bottle in kit component list. • Updated kit descriptions in Overview chapter. • Updated run setup steps when using AVITI OS v3.3. • Updated expected priming time to 24 minutes. • Updated concentration of matrigel solution to 0.1–0.25 mg/ml. • Updated fixation reagent to 8% formaldehyde.
February 2025 Document # MA-00053 Rev. B	• Added well thumbnail images to show successful and unsuccessful results. • Added diagrams to sample preparation to emphasize proper pipette placement when adding or removing liquid from wells. • Reordered run preparation and setup steps to ensure flow cell is assembled before confirming flow cell assembly on the AVITI OS interface. • Updated step in fixation protocol to remove liquid from final wash before adding RNase inhibitor and storing fixed cells. • Updated microscope filter specifications to include nucleus (cy5) and membrane (cy3). • Updated volume of Teton optimization reagent from 80 µl to 70 µl. • Updated 1X PBS wash volume to 200 µl and 2 ml regardless of whether samples were stored before adding Teton reagents. • Updated note for optional permeabilization step to emphasize that the Element protocol does not require permeabilization.
December 2024 Document # MA-00053 Rev. A	• Initial release.

Technical Support

Visit the Documentation page on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

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EXHIBIT 19

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Specification Sheet

Element AVITI™ System

Unrivaled combination of cost, quality, and performance that fits any sequencing application at any scale

Highlights

- Multiple run starts daily
- Complete range of flow cells
- Exceptional accuracy with early insight into data quality
- Seamless compatibility with leading assays

Introduction

Next-generation sequencing (NGS) has revolutionized the field of genomics, empowering researchers to confront complex scientific questions with an evolving portfolio of technology and tools. Offering an unprecedented view of DNA, NGS fuels scientific discovery around the globe. Despite these innovations, the cost of benchtop sequencing has remained high, requiring factory-scale throughput to achieve any savings. A compromise on cost is often at the expense of quality and flexibility. Many labs turn to outsourcing, conceding delays in pursuit of lower costs.

To overcome these tradeoffs and drive more science, the Element AVITI System reimagines the core components of NGS to offer a benchtop platform that grants access to the genomics ecosystem (Figure 1). Delivering flexible throughput at exceptionally low cost, the AVITI System saves time and resources without the need to batch or accept lesser quality. Avidity Sequencing™ forms the core of a disruptive design that readily adapts to any application, offering methods that scale from amplicon to whole genome, and from short-read to long.

Scalable experimental design

Whether an experiment needs 2 billion reads per run or only 100 million, the AVITI System enables cost-effective, high-quality sequencing across a broad scale. Multiple sequencing kit configurations from read lengths of 2 x 75 to 2 x 300 and a full range of high-, medium-, and low-outputs calibrate genomic output without sacrificing cost-effectiveness, even at small scales (Table 1). The kits support a range of insert sizes while accommodating unique dual indexes (UDIs) and unique molecular identifiers (UMIs).

Figure 1. An AVITI System dramatically reduces sequencing costs and turnaround times while elevating the benchmark for sequencing data, all in a compact benchtop format that fits into a variety of spaces.

Individually addressable lanes exert more control over samples and timelines, providing the ability to isolate a library pool in a single lane or sequence two library pools on one flow cell without additional sequencing kits.

Rapid Cloudbreak™ chemistry

Cloudbreak chemistry advances the core Avidity Sequencing technology with increased accuracy, efficiency, and speed. In only 38 hours, two 2 x 150 runs with indexing generate ≤ 600 Gb of data and 2 billion reads. These accelerated turnaround times maximize potential sequencing output during a regular workday, allowing daily completion of up to two 2 x 75 runs.

Industry-leading performance

The AVITI System resets expectations on quality scores (Q-scores), at ≤ 300 cycles delivering the most accurate specification available today with > 90% of bases scoring Q30.[1] A 2 x 300 kit achieves > 80%. Q-scores exceeding Q40 are routine. An assessment of data quality concluded that across all 20–50x coverages, the AVITI System demonstrated higher accuracy compared to legacy sequencing technology. AVITI System data had fewer soft-clipped reads in difficult homopolymer and repeat regions, among other clear advantages.[2]

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Read Length	High Output Kit (Gb/hours)[a]	Medium Output Kit (Gb/hours)	Low Output Kit (Gb/hours)
Read Count	*1 billion*[b]	*500 million*	*250 million*
2 x 75	150/24	75/20	Not applicable
2 x 150	300/38	150/31	75/27
Read Count	*300 million*	*100 million*	*Not applicable*
2 x 300	180/60	60/51	Not applicable

[a] Individually addressable lanes slightly extend run times and produce the same output. Each lane contributes half the output.

[b] Performance metrics, including read counts, are based on sequencing Element-prepared libraries. Actual results might differ based on factors such as library type and preparation.

Table 2. Output specifications for the AVITI System

Innovative sequencing chemistry

The fundamentals of Avidity Sequencing translate into real-world benefits for data quality and value. The chemistry leverages the unique properties of avidites to execute an efficient sequencing reaction that yields highly accurate data.[3] A primary driver of this accuracy is a strong signal-to-noise ratio that persists through high polony densities.

At the start of a run, the library hybridizes to surface primers coating the flow cell. Amplification polymerase then binds to the library and primer duplexes, catalyzing rolling circle amplification (RCA) and generating long DNA strands that include copies of the original library. Each strand forms a polony that contains hundreds of copies of the original library. The polonies hybridize to read-specific sequencing primers.

A cycle begins with a sequencing polymerase binding an avidite to a polony and primer duplex, trapping a base-specific avidite to the polony for imaging and forming an extremely tight complex that enables a 100-fold reduction in reagent concentration compared to sequencing-by-synthesis, by extension driving down the cost per sample (Figure 2). After imaging, the avidites are removed and unlabeled nucleotides are incorporated into the sequencing primer to extend the primer by one nucleotide. Another cycle begins.

Amplification advantages

RCA uses only the original strand as a template to avoid magnifying amplification errors. This amplification method also limits the effects of index hopping and optical duplicates:

- Index hopping assigns reads to the wrong sample and is most pronounced on high-throughput systems using non-RCA amplification. RCA avoids incorporating free index primers into polonies and minimizes index hopping on the flow cell.
- Optical duplicates occur when the software attributes sequences from one large polony to two smaller polonies and separately computes the calls. A low rate of optical duplicates—the rate for the AVITI System is < 1%—results in a greater number of usable reads.

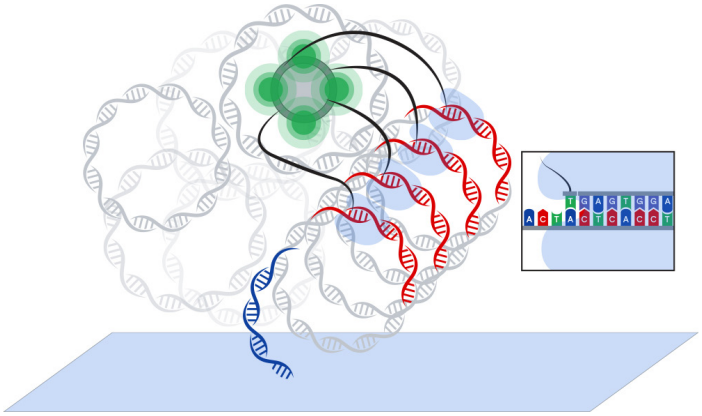

Figure 2. Polymerase binds avidites, trapping them at the incorporation site of template DNA. The avidite arms connect to a core that provides a fluorescent signal for detection. Low-binding surface chemistry makes the signals appear more prominent against a dark backdrop.

Complete NGS solution

The AVITI System grounds an end-to-end NGS workflow that integrates library prep, sequencing, and analysis (Figure 3). Partnerships with a growing range of library prep and analysis companies validate Element solutions and facilitate the transition to the AVITI System. Fixed reagent pricing for the lifetime of the instrument provides assurance for future operational costs and neutralizes batching requirements to expedite results.[4]

Any library prepared with the Element Adept™ Library Compatibility Workflow or Element Elevate™ Library Prep Workflow is compatible with the AVITI System. Both workflows offer robust library prep with broad input requirements and serve as the main entry point for sequencing on the AVITI System. The key difference is methodology: the Adept Workflow adapts existing libraries and the Elevate Workflow prepares libraries from input DNA. 16S LoopSeq™ for AVITI and Amplicon LoopSeq for AVITI provide specialized library prep solutions that are also compatible with the AVITI System.

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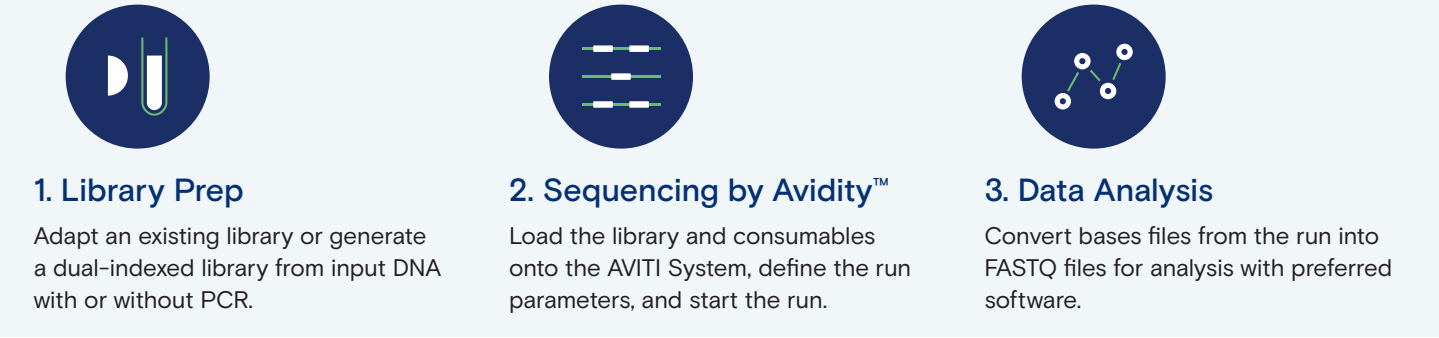

Figure 3. The AVITI System seamlessly integrates genomics resources to offer a sequencing workflow that balances ease of use with the freedom to refine experiments for specific research needs. The AVITI System is compatible with the Adept Workflow, Elevate Workflow, and LoopSeq for AVITI. Bases2Fastq generates FASTQ files.

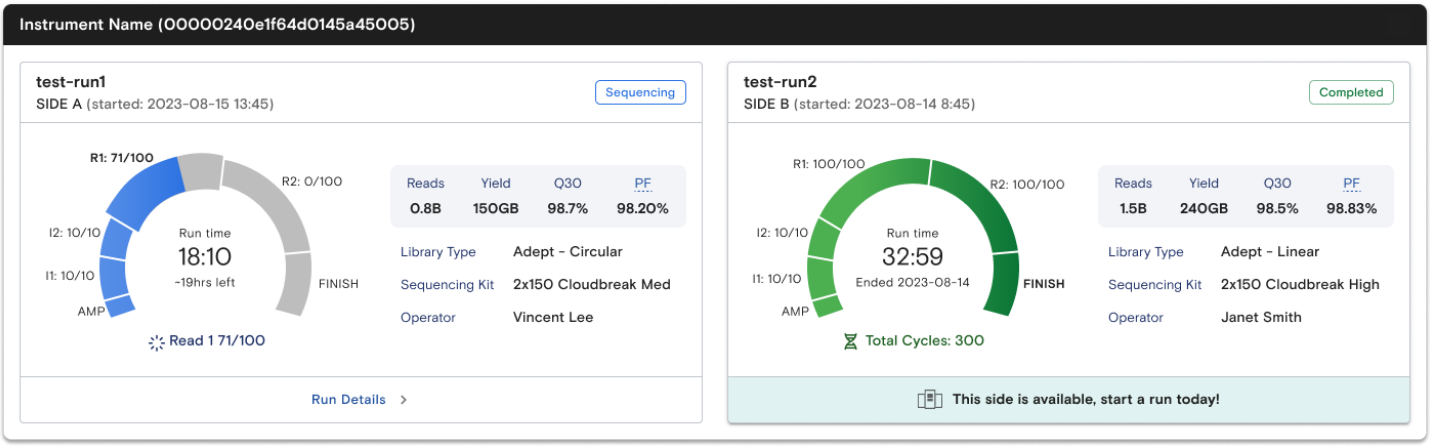

Figure 4. Elembio™ Cloud is an online platform for managing instruments, monitoring runs in real time, and visualizing run metrics to gauge performance.

Adept Workflow for adapted libraries

The Adept Workflow adds Element sequences to linear libraries prepared with a compatible third-party library prep kit. This automation-friendly workflow supports custom primers and allows labs to continue using the same library prep and analysis tools with the AVITI System. Highly accurate quantification optimizes polony density, in turn improving data quality and output.

Optional amplification makes the ends of any incompatible or potentially incompatible library compatible. For an up-to-date list of supported kits, visit go.elembio.link/compatible.

Elevate Workflow for native prep

The Elevate Workflow prepares linear libraries for whole-genome sequencing (WGS). This straightforward workflow integrates with Cloudbreak chemistry to automatically circularize libraries onboard the instrument as part of the run, minimizing hands-on time. A modular kit design enables end-to-end library prep with mechanical or enzymatic fragmentation and the option to integrate Elevate indexes and adapters with a preferred third-party library prep. PCR-free and PCR-plus protocol options round out this flexible WGS solution.

LoopSeq for AVITI bundles Elevate and LoopSeq to generate AVITI-ready libraries with Elevate indexes and adapters. This pairing brings long-read capability to the AVITI System.

Simple and secure data analysis

AVITI Operating Software (AVITI OS) allows you to specify the ideal storage location, keeping genomic data exclusively in your hands.[5] Guided workflows step through run setup with helpful reminders, menu-style selection of run parameters, and consumable validation. An intuitive user interface guides run setup, run monitoring, and system configuration from the instrument. Elembio Cloud extends AVITI OS capabilities, offering a similarly intuitive view with a rich set of real-time run metrics explorable through a computer or mobile device (Figure 4).

Throughout a run, the software analyzes images and uses the data to call bases and assign Q-scores. These data are packaged into bases files that serve as input for Bases2Fastq Software, which generates FASTQ files for analysis in a preferred application. Features that detect and apply the correct index sequence orientation and automatically identify and trim adapter sequences eliminate guesswork.

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Figure 5. A centralized touchscreen monitor (A) simplifies operations. Nests hold two flow cells (B), one for each side, and an LED display (C) communicates the status of a side. The reagents (D) and waste bottles (E) smoothly load and unload from the instrument.

Real-time run QC

An index-first run format sequences the Index 1 and Index 2 reads before the DNA insert, allowing early demultiplexing onboard the instrument for early insight into index assignment metrics, providing confirmation of a high-quality run or sparing the time of a low-quality run. Further downstream, Bases2Fastq detects and applies the correct index sequence orientation for virtually error-proof FASTQ file generation without the guesswork. A related adapter detection feature automatically identifies and trims adapter sequences.

Efficient instrument layout

The AVITI System is a compact benchtop instrument that suits a variety of spaces (Figure 5). Each side of the instrument—side A on the left and side B on the right—is dedicated to one flow cell and operates independently. This dual-sided layout essentially places two systems on the benchtop for the price of one. Moreover, the system ships with accessories designed to minimize waste and facilitate disposal of hazardous reagents.

Dedicated service and support

A dedicated and experienced Element team simplifies service and support and help keep the system operating at peak performance with minimal interruptions. The fully staffed team includes field service engineers to support site prep and installation and verify the system, field application scientists to remove technical barriers and host scientist-to-scientist conversations, and additional engineers and scientists to provide rapid phone and email support.[6]

System sensors measure the performance of key components and send instrument health data to Element. This onboard telemetry rewards labs who partner with Element for proactive system maintenance. Enabling telemetry automatically shares a curated set of metrics that identify potential problems early. The reports are carefully configured to protect sensitive information and do not include any sequencing data. The Element service team cooperates with labs to address any problems quickly and with minimal disruption.

Sequencing at your scale

An alternative model of the full-throughput AVITI System, the AVITI LT runs low- and medium-output sequencing kits to offer low-throughput and budget-friendly access to Avidity Sequencing. If future growth and expanded applications require a broader range of throughputs, labs can easily update the AVITI LT to an AVITI System, which runs all kits. Multi-system labs with high volume can leverage the $200 Genome Program to sequence at as little as $200 per genome or $2 per Gb.

System specifications

Instrument Configuration
Dual flow cells
AVITI Operating Software with a touchsceen display
Ubuntu Core 20.04 LTS operating system

Operating Environment
Temperature: 18–26°C
Elevation: < 2000 m
Sound level: ≤ 62 db at 3.3 ft

Instrument Dimensions
(H x W x D) 29.5 in x 37.6 in x 28.5 in
Weight: 155.1 kg/342 lb

Crate Dimensions
(H x W x D) 48.6 in x 51 in x 35 in
Weight with instrument: 245.9 kg/527 lb

Power Requirements
100–240 VAC at 50/60 Hz, 15 A. 550 W (average)

Summary

The AVITI System reinvents surface chemistry, base detection, and data analysis to offer a flexible and cost-effective sequencing platform that readily supports a variety of NGS applications. From the AVITI LT to the $200 Genome Program, the AVITI System grows with your needs. Overarching compatibility with standard NGS libraries provides a path to in-house sequencing while integrated and user-friendly software tools streamline operations. Multiple kits at locked prices and abundant software features promote adaptive run setup and analysis to satisfy a spectrum of experiment needs without the demands of batching.

Ordering information

Product	Catalog #
Element AVITI System	880-00001
Element AVITI System LT	880-00003
AVITI 2x75 Sequencing Kit Cloudbreak Medium Output	860-00007
AVITI 2x75 Sequencing Kit Cloudbreak High Output	860-00004
AVITI 2x150 Sequencing Kit Cloudbreak Low Output	860-00005
AVITI 2x150 Sequencing Kit Cloudbreak Medium Output	860-00006
AVITI 2x150 Sequencing Kit Cloudbreak High Output	860-00003
AVITI 2x300 Sequencing Kit Cloudbreak Medium Output	860-00009
AVITI 2x300 Sequencing Kit Cloudbreak High Output	860-00008
Adept Cloudbreak Custom Primer Set	820-00009

To learn more, visit elementbiosciences.com/products/aviti

References

1. Semyon Kruglyak, "Measuring the Accuracy of Element AVITI Sequencing Data," Element Biosciences (blog), July 13, 2022, https://www.elementbiosciences.com/blog/measuring-accuracy-element-aviti-sequencing-data.
2. Carroll, Andrew, Alexy Kolesnikov, Daniel E. Cook, et al., "Accurate human genome analysis with Element Avidity sequencing," *bioRxiv* (August 2023): https://doi.org/10.1101/2023.08.11.553043.
3. Arslan, Sinan, Francisco J. Garcia, Minghao Guo, et al., "Sequencing by avidity enables high accuracy with low reagent consumption," *Nature Biotechnology* (May 2023): https://doi.org/10.1038/s41587-023-01750-7.
4. "Reagent Price Guarantee - Announcement Video," Element Biosciences, accessed February 27, 2023, https://www.elementbiosciences.com/resources/our-story/our-mission/reagent-price-guarantee-announcement-video.
5. Element Biosciences, *Elembio Cloud and Element AVITI System Data Protections White Paper*, July 2023, doc. no. MA-00012.
6. Element Biosciences, *Element AVITI System Site Prep Guide*, October 2022, doc. no. MA-00007.

Element Biosciences

Telephone: 619.353.0300
Email: info@elembio.com

EXHIBIT 20

Element Biosciences

AVITI24™ System

User Guide

FOR USE WITH

AVITI24 System, catalog # 880-00004
AVITI Operating Software v3.3.0 or later

ELEMENT BIOSCIENCES
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Document # MA-00051 Rev. D
April 2025

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Table of Contents

CHAPTER 1

System Overview

Introduction

The AVITI24 System is a multidimensional genomics instrument that integrates state-of-the-art sequencing with cytoprofiling in one benchtop platform. The AVITI24 System measures a diverse array of molecular features for deep multiomic profiling of RNA, protein, and morphology in a single sample in 24 hours.

The AVITI24 System offers flexibility through multiple sequencing kit configurations and Teton™ cytoprofiling kits that support an extensive range of applications. The dual flow cell design enables parallel runs or staggered runs with independent run setup options. AVITI Operating Software (AVITI OS) provides an abundance of additional features to promote adaptive run setup and streamline analysis.

This guide provides an overview of system components, analysis options, maintenance instructions, configuration settings, and safety information for the AVITI24 System.

Site Prep and Safety

Before installation of an AVITI24 System, ensure your site meets the requirements in the *AVITI24 System Site Prep Guide (MA-00052)*. Before operating or maintaining the instrument, review the safety and regulatory information in *Safety and Compliance* on page 58.

The instrument does not contain any user-serviceable parts. Exterior shells enclose the instrument to protect the operator from laser light exposure and mechanical parts. Software and interlocks prevent exposure to hazards, and using the AVITI24 System in an unspecified manner can compromise these protections.

Warranties and Services

The purchase of an AVITI24 System includes a standard one-year warranty. Element offers supplemental procedures, preventative maintenance service, and annual service plans. For more information, visit elementbiosciences.com/instrument-service-coverage.

System Compatibility

For sequencing runs, the AVITI24 System is compatible with single-strand DNA (ssDNA) libraries prepared with particular library preparation workflows and that use Element sequencing chemistry. For more information on compatibility, see the *Product Compatibility* page on the Element website.

To avoid mixing and matching components from different kit configurations and versions, AVITI OS validates the compatibility of the cartridge and flow cell provided in each sequencing or cytoprofiling kit.

Additional Documentation

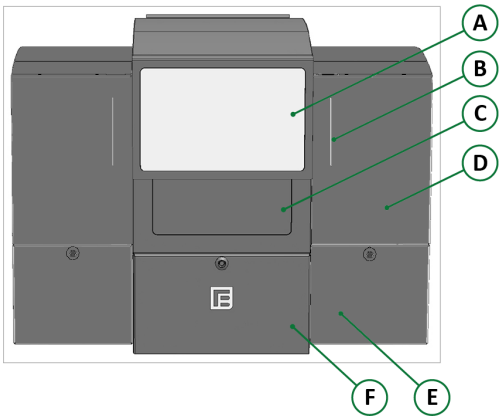
For run preparation and setup instructions for a specific workflow, see the following guides:

- *Cloudbreak Sequencing User Guide (MA-00058)*
- *Trinity Sequencing User Guide (MA-00059)*
- *Teton CytoProfiling User Guide (MA-00053)*

AVITI24 System Components

The instrument is divided into two sides, side A on the left and side B on the right when facing the instrument. Each side operates independently so you can engage one side while the other is in use. Side A and B each include a dedicated pump bay and reagent bay enclosed with bay doors.

Between sides A and B is the glove-compatible touchscreen monitor that displays the AVITI OS interface. Below the monitor is the nest bay and the waste bay. Lighting illuminates the interior of each bay. During a run, AVITI OS locks all doors except the pump bay doors to protect against laser light exposure, mechanical moving parts, and other hazards.



- **A** Touchscreen monitor
- **B** Lightbars
- **C** Nest bay with automated nest door
- **D** Pump bays hold fluidic pumps
- **E** Reagent bays hold reagents for each run
- **F** Waste bay holds waste bottles

CAUTION
Do not place items on top of the instrument or on open doors. The doors can support the weight of run and wash components, but applying extra weight or bumping into an open door can damage the instrument.

Status Lights

The AVITI24 System includes two types of status lights: an interior nest light in front of each nest and an exterior lightbar on each side. The nest light colors indicate flow cell status. The lightbar colors indicate the current process and overall system status. Unless the system is initializing, each lightbar is side-specific.

Nest Light Colors

Color	Status
Blue	The flow cell is present and ready to be unloaded.
Green	The flow cell is properly loaded and ready for priming, sequencing, or washing.
Red	The flow cell is improperly loaded: the lid is open or the nest is empty.
None	The flow cell is present but is not ready to be unloaded.

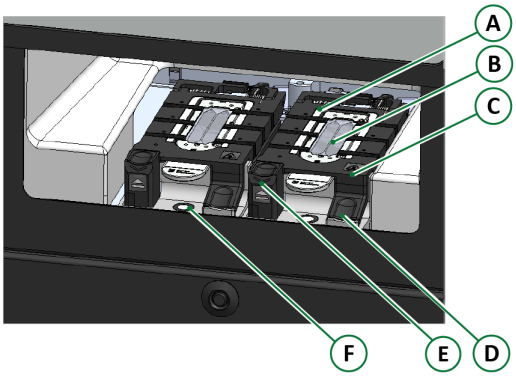
Lightbar Colors

Color	Status
White fade	The system is initializing.
Solid white	The system is initialized and idle.
Solid blue	Run or wash setup is in progress.
Blue fade	The system is priming, sequencing, or washing.
Solid orange	The system experienced a warning. The color changes after the run finishes.
Solid red	The system experienced an error or run failure. The color immediately changes when an error occurs.

Nest Bay

The nest bay includes two nests, one for each side, and each nest holds one flow cell. A hinged flow cell lid secures the flow cell in place. A button on each nest unlatches and opens the hinged lid to a 40° angle. To ensure proper alignment, three silver pins on the loading area fit into three corresponding holes on the flow cell cartridge.

An automated nest door on the middle shell encloses the nest bay. During a run, a camera and four tube lenses above the nest image the flow cell in four channels.

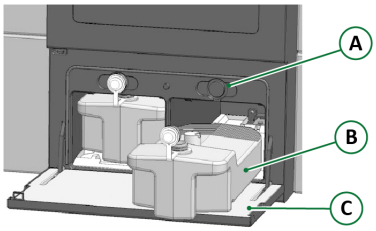


A Wall around the loading area
B Flow cell loaded on the nest
C Lid secured over a loaded flow cell
D Tab to close the lid
E Button to open the lid
F Nest status light

Waste Bay

The waste bay holds two waste bottles, one for each side. Two threaded cap holders above the waste bay secure the tethered transport cap to keep the caps clear of the door.

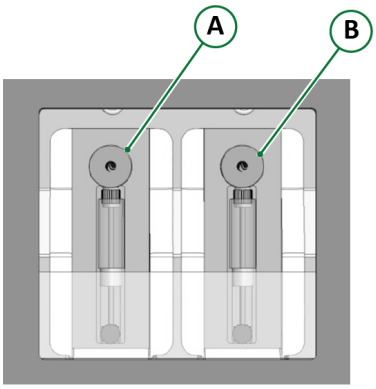
A sealed tray built into the bottom of the waste bay collects spills and leaks and directs liquid to the front of the instrument. During run or wash setup, sensors confirm the waste bottle is present and empty and allows the run or wash to proceed. Another sensor detects spills.



A Cap holder
B Waste bottle
C Open waste bay door

Pump Bays

Each pump bay contains two pumps that control the flow of liquid. The left pump pulls fluid through the left lane of the flow cell and the right pump pulls fluid through the right lane. Keep the pump bay doors, which allow service access, closed during normal operation and maintenance.

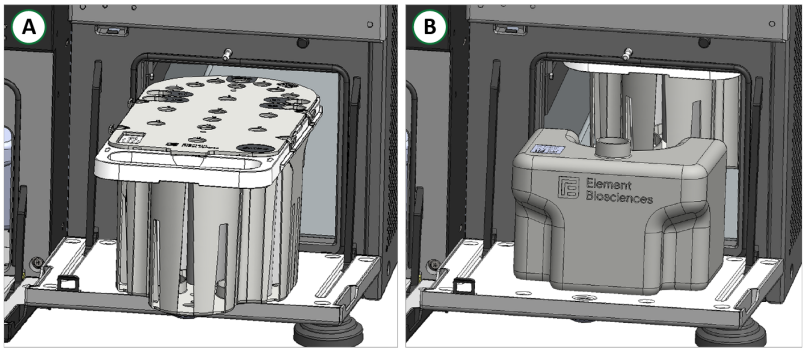


A Pump controlling the left lane of a flow cell
B Pump controlling the right lane of a flow cell

Reagent Bays

Each reagent bay holds a buffer bottle and cartridge basket that contains a cartridge or a wash tray, depending on whether the system is sequencing or washing. Keep the reagent bay doors closed to maintain the refrigeration, which chills reagents.

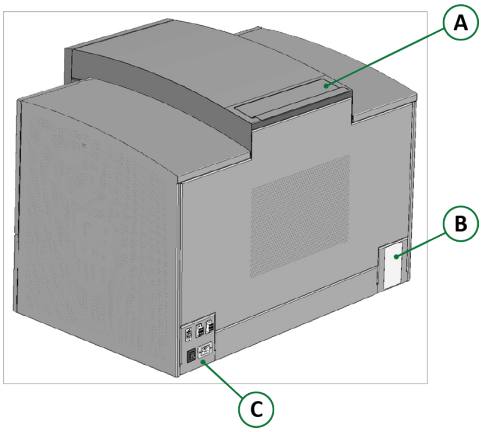
When priming starts, sippers descend into the bay, pierce the foil seals covering the cartridge wells, and aspirate reagents from the bottom of each well. The sippers continue to aspirate reagents throughout the run. Functioning similarly for a wash, the sippers aspirate wash solution instead of reagents.



A Loading a basket and cartridge
B Loading a buffer bottle

Back Panel

The back panel includes the air filter tray and input and output (IO) panel. A compliance label displays regulatory symbols for regulatory compliance, the instrument serial number, and electrical specifications. For more information on labeling, compliance, declarations, and certifications, see *Safety and Compliance* on page 58.



A Air filter tray
B Compliance label
C IO panel

Air Filter Tray

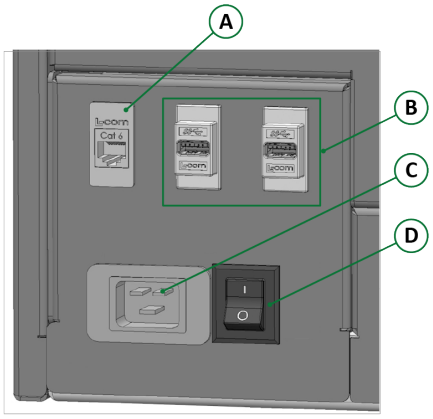
Air enters the instrument through a disposable air filter constructed of pleated paper. The air filter is rated MERV 8, which keeps dust out of the instrument but does not filter smoke or particles < 3 microns. Keeping aerosol and particulate sources away from the instrument extends filter life.

A tray that lifts out of the top of the instrument contains the air filter and facilitates easy replacement. For instructions, see *Replace the Air Filter* on page 35.

Input and Output Panel

An IO panel on the back of the instrument groups connections and the power switch. A Category 6 (Cat6) Ethernet port connects an Ethernet cable, and a power entry module connects the power cord. When connecting the instrument to power, use only the power cord that Element provides.

The IO panel also includes two USB 3.0 ports to connect a mouse, keyboard, or drive for transferring files. Side B includes a third USB 3.0 port. A USB drive that transfers files to or from the instrument must be in ***FAT32 format***.



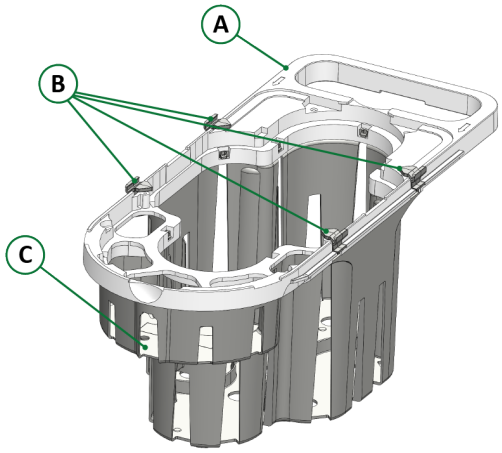
A Cat6 Ethernet port
B USB 3.0 ports
C Power entry module
D Power switch in the on position

Reusable Accessories

Cartridge baskets, wash trays, and waste bottles support run setup and washes while minimizing waste. These accessories are reusable but require periodic replacement.

Cartridge Basket

The cartridge basket protects the cartridge during a run. The back of the basket extends into a handle with arrows that indicate the loading direction. Clips along the top of the basket secure the cartridge. The curved area under the handle accommodates the buffer bottle, which is loaded into the reagent bay behind the basket. A window at the front of the basket enables library inspection.



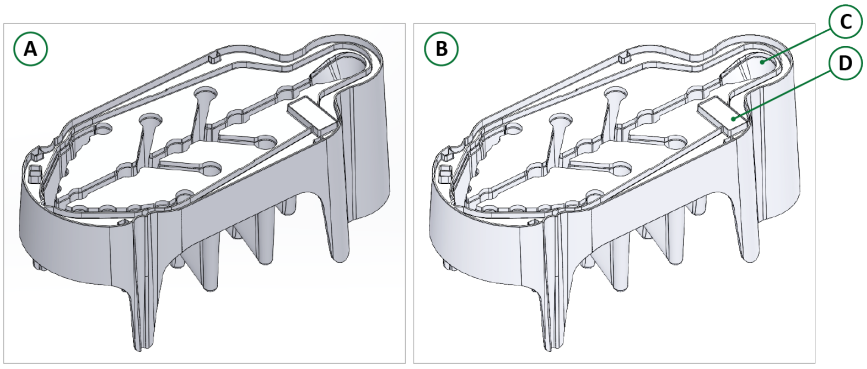
A Handle at the back
B Latches along the top
C Window at the front

Wash Trays

The AVITI24 System includes two types of wash trays, each dedicated to different wash solutions:

- AVITI Wash Tray 1, Gray, for use with Wash 1 Solution.
- AVITI Wash Tray 2, White, for use with Wash 2 Solution and nuclease-free water.

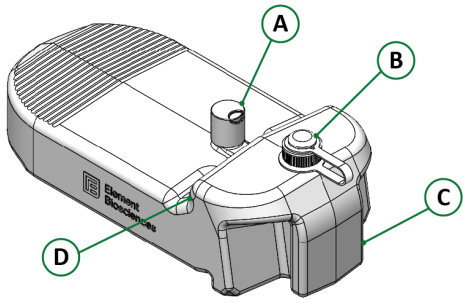
The back of a wash tray forms a handle with a fill area for adding wash solution. Interior fill lines indicate approximate volumes, and an overflow wall contains any wash solution that exceeds the 800 ml maximum fill volume. Each tray includes a water-proof barcode label for validation purposes.



A Gray wash tray
B White wash tray
C Handle with fill area
D Barcode label

Waste Bottle

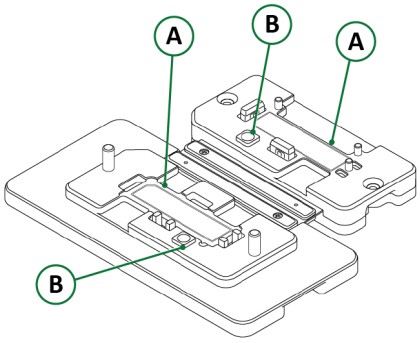
A waste bottle collects spent reagents and library throughout the run. The maximum capacity of 3.2 L per bottle is sufficient to contain all waste from one run. The tethered transport cap seals the bottle during transport. The vent cap improves flow when emptying waste. Ridges on the back of the bottle and a handle at the front facilitate handling.



A Vent cap
B Transport cap
C Handle
D Thumb indentations

Teton Flow Cell Aligner

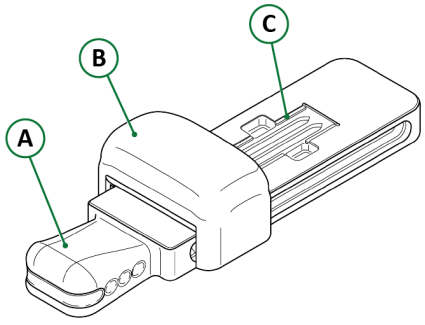
The Teton Flow Cell Aligner is used to combine the prepared sample slide from the slide kit with an adhesive slide from the flow cell assembly kit. After the slides are in position, the hinged fixture is designed to flip the adhesive side against the sample side, affixing the slides together. The affixed slides are then assembled into a flow cell cartridge.



A Indentation for slide placement
B Button to release slide holder

Teton Flow Cell Sealer

The Teton Flow Cell Sealer ensures complete adhesion of the sample slide and adhesive slide. An indentation in the tray secures the assembled slides as you slowly push the roller grip forward and back to remove any trapped air.



A Base grip
B Roller grip
C Indentation for slides

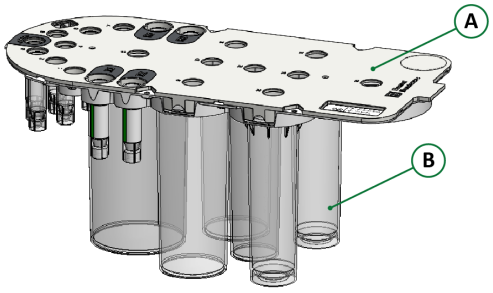
Sequencing Kits

A sequencing kit provides a flow cell, a reagent cartridge, loading buffer, and wash buffer required for one run. The cartridge for each kit supports a specific number of cycles and output levels. Components includes a barcode label for tracking and validation. To ensure the compatibility of run components, see the *Product Compatibility* page on the Element website.

Sequencing Cartridge

The reagent cartridge is a collection of reagents and buffers in foil-sealed wells that are packaged in an easy-to-load container. The cartridge lid secures the wells and labels the reagent positions. Each well is transparent to allow visual inspection after thawing. A barcode label enables tracking and validation.

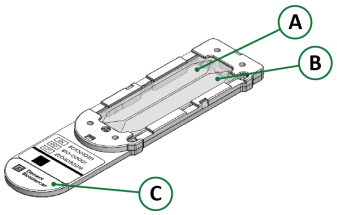
The Library well is reserved for the diluted library. For kits that are compatible with the Individually Addressable Lanes add-on, the AUX well is reserved for a second library. For more information, see *Individually Addressable Lanes* on page 19.



A Cartridge lid
B Transparent well

Sequencing Flow Cell

The flow cell is a two-lane glass substrate encased in a plastic cartridge. The cartridge includes a gripper for safe handling. Proprietary surface chemistry coats the flow cell and enables polony generation and sequencing. Library and reagents enter the flow cell through inlet ports and exit as waste through outlet ports.



A Lane 1
B Lane 2
C Gripper

Loading and Wash Buffers

A sequencing kit includes multiple loading and wash buffers that are packaged separately. Instrument Wash is included in the sequencing cartridge.

Buffer	Packaging	Description
Library Loading Buffer	Tube	The reagent for diluting the libraries to the target loading concentration
AVITI Universal Wash Buffer	Buffer bottle	The reagent that flushes reagents from the flow cell during a run
Instrument Wash	Cartridge	The wash solution for the automatic post-run wash

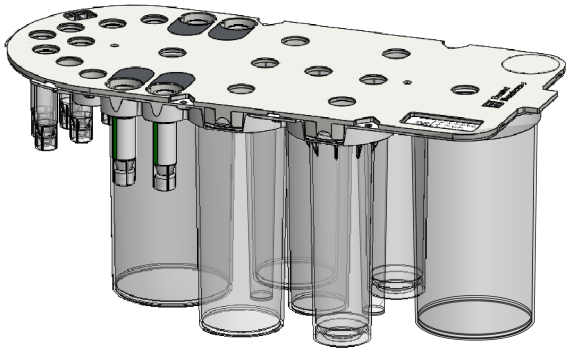
Cytoprofiling Kits

Kits for a cytoprofiling run are packaged as a reagent kit, a slide kit, and a flow cell assembly kit. Teton reagents support the identification of different cell targets, including cell paint targets, protein and transcript targets, and cell morphology.

- **Teton Reagent Kits**—Each kit includes a Teton reagent cartridge, a buffer bottle, and a Teton reagent kit. One Teton kit provides cytoprofiling and cell paint reagents.
- **Teton Slide Kits**—Slide kits are used to prepare samples for a run or for use with the Teton Optimization Kit for early assessment of your sample.
- **Teton Flow Cell Assembly Kits**—Flow cell assembly kits are required to load one sample slide for a cytoprofiling run.

Reagent Cartridge

The reagent cartridge is a collection of reagents in foil-sealed wells that are packaged in an easy-to-load container. The cartridge lid secures the wells and labels reagent positions. Each well is transparent to allow visual inspection after thawing. A barcode label enables tracking and validation.



Wash Buffers

Cytoprofiling kits include a wash buffer that is packaged separately and labeled with a barcode for tracking and validation purposes. Instrument Wash is included in the reagent cartridge.

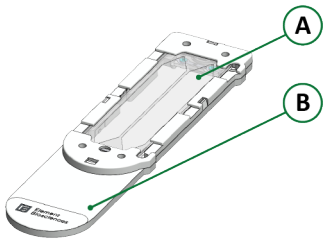
Buffer	Packaging	Description
AVITI Universal Wash Buffer	Buffer bottle	The reagent that flushes reagents from the flow cell during a run
Instrument Wash	Cartridge	The wash solution for the automatic post-run wash

Teton Slide Kit

The Teton workflow requires a Teton Slide Kit for preparing samples directly onto the slide. The slide later becomes part of the Teton flow cell. The slide kit includes a barcode for tracking and validation. For more information, see the *Teton CytoProfililng User Guide (MA-00053)*.

Teton Flow Cell Assembly

The Teton flow cell is a combination of two slides affixed together. The affixed slides are assembled into a plastic cartridge with flow cell port gaskets provided in the Teton flow cell assembly kit.



A Combined slides

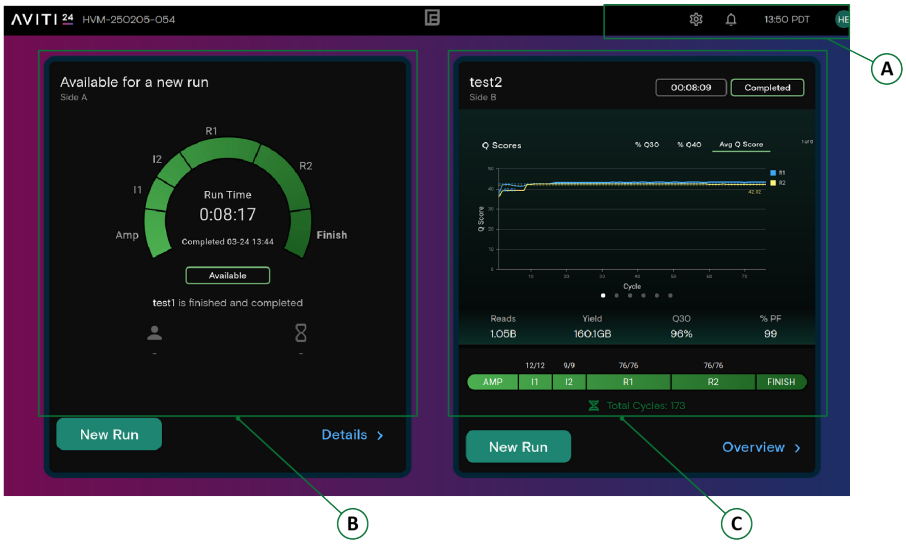
B Assembled flow cell cartridge

CHAPTER 2

Software and Analysis

AVITI Operating Software

AVITI OS controls instrument operations during sequencing, analysis, and instrument washes. The Home screen functions as a system dashboard, displaying the status of each side with features to start runs and washes and monitor runs.



A Taskbar
B Overview view
C Details view

Home Screen Views

The Home screen includes buttons that display the following views:

- **Overview**—Displays general system status and previous run times for each side, or displays active run or wash information.
- **Details**—Displays metrics for an active run. When a run starts, AVITI OS automatically switches to this view.
- **History**—Preserves metrics from the last run. When no run or wash is active, this view is available.

Taskbar Icons

A taskbar at the top of the Home screen provides the following icons. The Settings and Notifications icons each open a unique screen. USB Drive and User icons each access additional features and functions.

Icon	Name	Function
	USB Drive	View a list of USB drives that are connected to the instrument and safely disconnect a USB device.
	Settings	View system information and configuration settings. See *Settings* on page 18.
	Notifications	Review notifications and perform the indicated action. See *Notifications* on page 20.
	User	Open the User menu. Alternatively, this icon displays initials.

Settings

AVITI OS includes configurable and read-only settings that control the instrument profile and system connections. AVITI OS divides the settings among the following tabs:

- **About**—Displays software and instrument information:
  - » AVITI OS version and the last license acceptance date
  - » AVITI24 System name, instrument type, serial number, available local storage, and compute ID
  - » Updates available for system firmware and software

  NOTE
  Compute ID is a unique code for the integrated circuit that identifies the instrument computer.
- **General**—Controls the system name and displays on-screen keyboard, telemetry, and elevation settings. Also, exports log files from offline systems and resets the air filter time.
- **Network**—Controls network and internet connections for the system. Includes a connectivity indicator.
- **Storage**—Lists storage connections with connectivity indicators and settings for adding and managing storage connections.
- **Add-Ons**—Displays the add-ons enabled on the system and any applicable expiration dates. The tab always appears on offline systems and only appears on online systems with at least one active add-on.
- **User**—Provides password management for offline systems and online systems with local authentication. The tab only appears on applicable systems.

Network Status

The Network tab displays the following icons, which indicate the status of the network connection. An additional Indicator appears on the tab to show internet connectivity.

Icon	Network Status
	Connected
	Local internet only
	Disconnected

Storage Status

The Storage tab displays the following icons, which indicate the status of the storage connections. An additional Indicator appears on the tab to show storage connectivity.

Icon	Storage Status
	At least one storage connection
	No storage connection

Add-Ons

Add-ons enable additional instrument capabilities. The Add-Ons tab displays each add-on available on the instrument. To enable an add-on, contact a sales representative or Element Technical Support.

For online instruments, AVITI OS refreshes the add-on list every 12 hours and when the system restarts. For offline instruments, the installation of add-ons requires additional steps. For more information, see *Install Add-Ons on an Offline System* on page 49.

Filter Mask

The Filter Mask add-on modifies the cycles used for filtering, which is advantageous for certain applications. Applying the feature causes run output data and on-instrument run metrics to account for the filter mask.

High Output Kits

The High Output Kits add-on enables sequencing with high-output kits.

Individually Addressable Lanes

The Individually Addressable Lanes add-on enables loading one library pool in each lane of a flow cell for sequencing runs. The second library is loaded into the AUX well of the sequencing cartridge.

The add-on is only compatible with sequencing kits that meet the following requirements:

- Cloudbreak™ or Cloudbreak Freestyle™ chemistry
- 2 x 75 or 2 x 150 size
- A high-, medium-, or low-output designation

CAUTION
The Individually Addressable Lanes add-on is ***not*** compatible with any 2 x 300 size sequencing kits, the Cloudbreak UltraQ™ sequencing kit, or Trinity sequencing kits. The cartridges in these kits reserve the AUX well for other reagents and cannot accommodate a second library.

PMG Shift

Polony map generation (PMG) refers to the process of mapping polonies during a sequencing run. The PMG Shift add-on enables the skipping of up to 20 cycles for compatibility with particular sequencing runs for Adept™ or third-party libraries. Skipped cycles do not affect data output. For more information on the applicability of this add-on, contact Element Technical Support.

ElemBio Catalyst

ElemBio Catalyst™ is a native cloud storage and analysis subscription service within ElemBio Cloud. ElemBio Catalyst allows Element to host and manage cloud storage connections on your behalf. Your data is stored in Amazon Simple Storage Service (Amazon S3) storage buckets that are completely dedicated to you.

To use ElemBio Catalyst, purchase an ElemBio Catalyst subscription or subscribe to a 45-day free trial. If your ElemBio Catalyst storage connection is disabled, an **Expired** badge is displayed for 14 days and the run storage connection cannot be used to upload runs. After 14 days, the ElemBio Catalyst storage connection is no longer visible in AVITI OS. To resubscribe to ElemBio Catalyst, contact Element Technical Support at support@elembio.com. For more information, see the *ElemBio Catalyst documentation* in *Online Help*.

Polony Density

The Polony Density add-on allows users to opt for an increased read output that is prioritized over the highest quality reads and lower error rates. When this add-on is enabled, it is available in the Advanced Run Settings form while you set up a run.

Notifications

Notifications display system messages across three tabs: General, Side A, and Side B. Expand a notification to see the message, date, and time.

Notification	Icon	Description	Action
Success		A run or wash completed successfully.	Acknowledge successful completion.
Information		The software is ready to be updated to a new version.	Acknowledge the update.
Warning		The system requires your attention, but you can continue operation.	Acknowledge the warning and resolve it by the indicated date.
Error		The system has malfunctioned and requires action to proceed.	Follow the onscreen prompt.

Unread Notifications

Notifications include badges that indicate the number of unread messages. Checkboxes mark notifications as read or unread. Marking a notification as read can reset the status lights on that side of the instrument.

Icon	Name	Action
	Mark as read	Mark the selected notifications as read.
	Mark as unread	Mark the selected notifications as unread.

Filtering and Sorting

Notifications include filters with sorting from newest to oldest or oldest to newest.

Filter	Description
All	View all messages on the selected tab.
Read	View only read messages on the selected tab.
Unread	View only unread messages on the selected tab.

Run Start Options

AVITI OS includes the following options for starting a run:

- **Single start**—Set up and start a run on one side of the instrument.
- **Dual start**—Concurrently set up and start runs of the same run type on both sides of the instrument.
- **Flexible start**—Set up and start a run or recovery wash on a side of the instrument while a run is active on the other side.

AVITI OS allows sequencing with different kits on each side. Because the sides share a camera, the setup of one run can increase the duration of the other run.

Flexible Start

Flexible start safely pauses the active run and initiates a run or recovery wash on the other side of the instrument. When setting up the second run, AVITI OS finds a safe pause point before proceeding. While the run is paused, set up and start a run or recovery wash on the other side. The runs on both sides proceed asynchronously. For a flexible start recovery wash, the run on the other side proceeds concurrently.

When you initiate flexible start, AVITI OS indicates the typical wait time for the current run step. Pausing the first run typically takes several minutes but can take as long as ~2 hours, depending on the run step. AVITI OS also includes options to cancel flexible start and resume the active run.

For more information on flexible start wait times, see the run setup instructions in the user guide for your sequencing or cytoprofiling kit.

Wash Setup Screens

Initiating a wash opens a series of wash setup screens that guide you through setting up a maintenance, standby, or recovery wash. Wash setup functions similar to run setup, but closing the door validates the wash tray presence.

Run Setup Screens

When you initiate a run, AVITI OS guides you through a series of run setup screens. Each screen provides a set of steps and indicates run setup progress. AVITI OS unlocks the reagent and waste bay doors at the appropriate steps and prompts the loading of consumables. Closing a door validates the presence of each consumable and scans the consumable barcode. The software presents an alert if consumables are expired. A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.

After the step to empty waste and reload the waste bottle, priming starts automatically. Priming prepares reagents for delivery and pumps air and reagents through a used flow cell and the fluidic tubes, preventing contamination between runs.

Run setup steps differ based on the type of kit you are using. For detailed run setup instructions, see *Additional Documentation* on page 6.

Advanced Run Settings

During run setup, selecting the Advanced Run Settings button displays settings for additional features that let experienced users modify primary analysis and run recipe configurations. Available features depend on your kit selection, run type, and available add-ons.

Feature	Description
Custom Recipes	Tailors a run execution in consultation with Element. A recipe governs the stages of a sequencing run, so custom recipes can impact specifications and increase run times. The setting provides two options for recipes: • **Preloaded recipe:** Select a recipe on the instrument, such as the short insert or long insert custom recipes. To ensure run compatibility, contact Element Technical Support. • **Uploaded recipe:** Element creates an encrypted, custom recipe package as a .rec file, which you upload from a USB. To obtain a .rec file, contact Element Technical Support.
Filter Mask	Sets the mask for the Filter Mask add-on. See *Filter Mask* on page 19.
PMG Shift	Sets the number of cycles skipped for the PMG Shift add-on. See *PMG Shift* on page 19.
Polony Density	Relaxes certain quality filters to increase the total number of polonies in a run. The setting has two options, with Standard as the default option. The High Density option increases the read output. This feature is also known as Expert Mode HD.

Signing In and Out

Signing in to AVITI OS requires the email address and password for your organization. The first time you sign in to AVITI OS after instrument installation or an update, you must accept the license agreement. A Logout option on the User menu signs you out.

If requested, Element can enable local authentication mode for an online system. This feature assigns a fixed user name and user-defined password to sign in.

Run Manifest

AVITI OS uses a run manifest as an input file that stores run information. The format of the run manifest differs between sequencing and cytoprofiling runs. After a run, AVITI OS provides the run manifest as an output file to support run analysis.

The run manifest uses a comma-separated values (CSV) file format and can be created using a template on the Resources page of the Element Biosciences website. For more information on creating a run manifest, see the *Run Manifest Documentation* in the *Online Help*.

Sequencing Run Manifest

For sequencing runs, the run manifest includes demultiplexing settings, settings for FASTQ files, and a list of samples with any corresponding index sequences. After a sequencing run, you can use the run manifest that AVITI OS provides to support secondary analysis and Bases2Fastq. Demultiplexing indexed libraries requires preparing a run manifest.

When a sequencing run does not include a run manifest, AVITI OS generates a default run manifest that assigns all reads to one sample during FASTQ file generation.

Demultiplexing indexed libraries is ***not possible*** with a default run manifest. To use a default run manifest with Bases2Fastq, you must edit the file and create a corrected run manifest that includes sample and index information.

Run Manifest for Sequencing with Individually Addressable Lanes

If you are using the Individually Addressable Lanes add-on, the Lane column in your run manifest must correctly associate samples with both library pools.

- Lane 1 refers to the library pool loaded into the Library well of the sequencing cartridge.
- Lane 2 refers to the library pool loaded into the AUX well of the sequencing cartridge.

For an example run manifest set up for the Individually Addressable Lanes add-on, see *Sample Specification Examples* in the *Online Help*.

Cytoprofiling Run Manifest

For cytoprofiling runs, the run manifest associates each well location with a label. AVITI OS and Cells2Stats can use these labels to refer to each well. Additional optional settings include the cell type and cell diameter.

When a cytoprofiling run does not include a run manifest, AVITI OS generates a default run manifest that uses the names of wells as well labels. The default run manifest does not include any optional settings.

Analysis Overview

Onboard primary analysis depends on the type of run you perform.

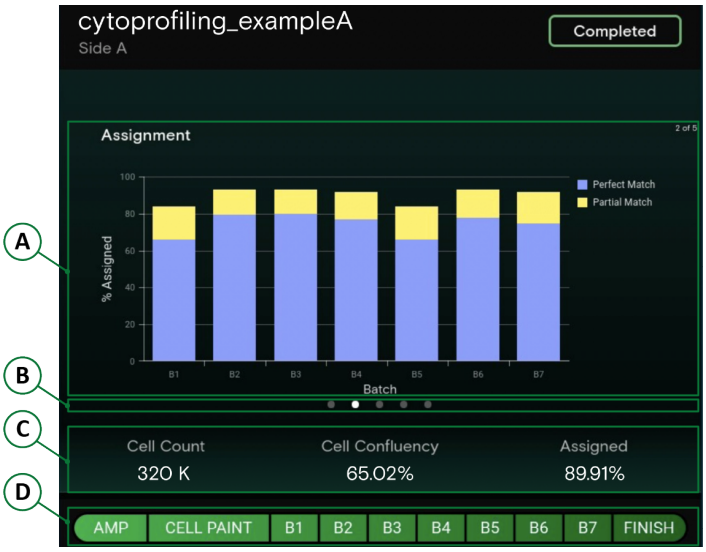
- **Sequencing runs:** AVITI OS calls bases, assigns quality scores (Q scores), and generates run metrics. The software extracts and corrects intensities from images to call a base, then assigns a Q score to the base.
- **Cytoprofiling runs:** AVITI OS uses cell paint images to segment cells and generate cell morphology features. The software also extracts and corrects intensities from images to call bases, count targets, and assign targets to cells.

Run Monitoring

During a run, the Details view displays initial estimates for primary-analysis-generated run metrics that monitor overall run health and progress. As the run progresses, metrics appear and regularly update. Sequencing runs that use the Individually Addressable Lanes add-on display metrics and charts for each library pool. The metrics are included in the run output and remain onscreen until you set up a new run.

The metrics and charts for a run depend on your workflow. Additional metrics appear in the charts, which you can cycle through. For more information on the types of metrics and charts that can appear during a run, see *Cytoprofiling Metrics and Charts* and *Sequencing Metrics and Charts* in the *Online Help*.

NOTE
To visualize cytoprofiling data with CytoCanvas, execute the Cells2Stats Software after the run completes. To obtain final metrics for a sequencing run, execute the Bases2Fastq Software after the run completes. For more information, see the *Cells2Stats Documentation* and *Bases2Fastq Documentation* in the *Online Help*.



A Example chart
B Carousel for available charts
C Example metrics
D Run progress information

Thumbnail Image

The thumbnail image displays a snapshot of the polonies on a tile from the run. For sequencing, the image is from the first cycle of the run. For cytoprofiling, the thumbnail displays the cell membrane and nucleus images from the cell paint batch. If you are performing a sequencing run with the Individually Addressable Lanes add-on and two library pools, AVITI OS displays an image for each pool.

The thumbnail image can serve as a quality check for your run. For sequencing, the image indicates sample density and loading concentration on the flow cell. For cytoprofiling, the image indicates the success of cell fixation.

Run Output and Data Transfer

The output of a run is a run folder. The folder contains files with data for the type of run. A storage connection transfers the run folder from the instrument to your local or cloud storage location. For more information, see *Storage Connections* on page 37.

Sequencing Run Output

The run folder for a sequencing run contains bases files with genomic data and other run data. Bases files are the primary output of a sequencing run. An AvitiRunStats.json file serves as the source file for run metrics. You can set up the integration of metrics into another system, such as a LIMS interface. For more information on output files, see *Sequencing Run Output Files* in the *Online Help*.

After a sequencing run, use Bases2Fastq to perform demultiplexing and convert the bases files into FASTQ files for secondary analysis with the third-party software of your choice. For more information, see the *Bases2Fastq Documentation* in the *Online Help*.

Cytoprofiling Run Output

The run folder for a cytoprofiling run contains images of cell paint targets, protein and transcript counts per cell, and cell morphology features. The data enable downstream cell analysis and characterization. You can use additional files to regenerate cell statistics and updated cell segmentation masks. For more information on output files, see *Cytoprofiling Run Output Files* in the *Online Help*.

After a cytoprofiling run, if necessary, you can use Cells2Stats to support cell resegmentation and additional analysis of your cell samples. For more information, see the *Cells2Stats Documentation* in the *Online Help*.

Local Disk Storage

Because the system software transfers runs to off-instrument storage locations, local disk storage is intended only for temporary storage. Accordingly, the instrument hard drive has sufficient space to store at least two runs and start an additional two runs. When you initiate run setup, AVITI OS checks whether the system has sufficient space to support the run. If AVITI OS indicates that the system does not have sufficient space, contact Element Technical Support.

Telemetry

Separate from the transfer of genomic data to your storage location, ***which Element cannot access***, telemetry sends instrument health data to Element. These data help support maintenance and troubleshooting and do not include any confidential information.

Telemetry is limited to the following data:

- **Software metrics**—Software and firmware versions, CPU and memory metrics, and the instrument serial number, ID, and name. These metrics are communicated as part of regular telemetry events.
- **Hardware metrics**—Data on motors, fans, lasers, and other instrument hardware, which helps Element understand the probable condition of select hardware components.
- **System logs**—Routine logs the system generates when idle or running. The logs include power cycle times, errors, internal communications, and the status of internal services.
- **Primary analysis metrics**—Run metrics, including data for Q30 scores, error rates, cell confluency, cell and target counts, expression levels, and index assignment metrics. Index assignment and other data exclude sample names.
- **Run information**—Data communicated for a run, including run name and ID, run side, run start and end dates and times, run type (sequencing, cytoprofiling, or washing), consumable information, and the number of cycles per read or batch. The data exclude run descriptions.

- **Run logs**—Run-specific information from a subset of system logs. Data include recipe execution, the timing of run steps, and communications between software, firmware, and hardware.

ElemBio™ Cloud

ElemBio Cloud is a central online platform that provides real-time remote run monitoring, data analysis, and account management for Element instruments, including AVITI24 Systems. Any system in online mode automatically connects to the platform. ElemBio Cloud allows you to connect to cloud service providers for data storage and initiate data analysis automation through flows.

You can access ElemBio Cloud on a computer or mobile device to support your organization from anywhere. For more information, see the *ElemBio Cloud Documentation* in the *Online Help*.

ElemBio Cloud Metadata

By default, AVITI OS sends the following metadata to a secure and customer-specific ElemBio Cloud database:

- Run description
- Sample names from the run manifest, if applicable

Metadata populate the run monitoring pages in ElemBio Cloud, which is separate from the telemetry database. Therefore, telemetry does not collect metadata. If you prefer to keep metadata on the instrument, contact Element Technical Support and request Restrict Metadata mode. When the mode is enabled, a lock appears on the run description in ElemBio Cloud and sample names are masked as numbers. The numbering reflects the order of samples in the run manifest.

CHAPTER 3

Maintenance

Maintenance Schedule

AVITI OS on the AVITI24 instrument requires a maintenance wash every 7 days. The software provides a warning 2 days before a maintenance wash is due.

Procedure	Frequency	Purpose
Maintenance wash*	Weekly	Cleans the outside of the sippers and prevents microbial growth and particulate debris from accumulating in the fluidic system.

Element recommends the following maintenance schedule for optimal performance.

Procedure	Frequency	Purpose
Power cycle	Weekly	Reinitializes the system and resets the instrument computer, which helps maintain instrument performance.
Standby wash*	Preparing for an idle period of ≥ 7 days	Prepares one or both sides for an idle period of ≥ 7 days.
Air filter replacement	Every 6–12 months	Ensures proper cooling and continuous operation. The optimum frequency depends on lab cleanliness.
Exterior cleaning	As needed	Wipe the exterior with a damp microfiber cloth and Simple Green. Avoid harsh chemicals and abrasives.

* To perform a wash after stopping a run, see *Stop an Active Run* on page 53.

For a list of maintenance consumables, see the *AVITI24 System Site Prep Guide (MA-00052)*.

Wash Tray Maintenance

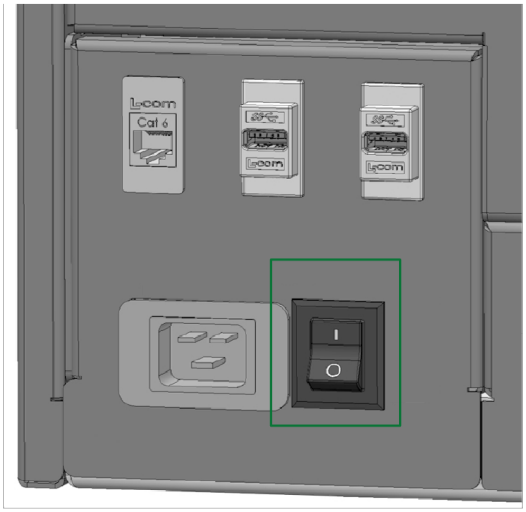
Keep the wash trays in good condition to maximize time between replacements and prevent cross-contamination.

- After each use, discard residual wash solution, rinse the wash tray with nuclease-free water, and air-dry upside down.
- Store clean and dry wash trays upside down. Stack up to two wash trays.

Power Cycle the System

A power cycle resets the instrument computer, safely shutting down and restarting the system to maintain performance or recover from a problem. Turning off the system without a proper power cycle is reserved for emergencies.

1. Select the user menu, and then select **Shut Down**.
2. When prompted, select **Shut Down** again to shut down the instrument computer.
3. Wait for the screen to go blank and a No Signal message to appear.
4. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.



5. Wait ***10 seconds*** to make sure the system fully shuts down.
6. On the IO panel, press the power toggle switch to turn on the instrument.

   —The system initializes and displays the Home screen.—
7. If a USB drive is connected to the instrument, reconnect it:

   a. In the taskbar, select **USB Drive**, and then select **Eject**.
   b. Detach the USB drive from the instrument.
   c. Reconnect the USB drive to the instrument.

   —Reconnecting the USB drive allows AVITI OS to detect it after a power cycle.—

Perform a Maintenance Wash

The maintenance wash is a two-part wash that takes a total of ~1.5 hours. Wash 1 cleans the system, removing residual library and carryover. Wash 2 rinses the system, removing residual Wash 1 solution and preparing for the next run. Each wash requires specific volumes of freshly prepared wash solutions.

Prepare Wash Solutions

1. Gather the following materials:
   - » 2 L bottles (2)
   - » 4.00–4.99% sodium hypochlorite
   - » Gray wash tray
   - » Nuclease-free water
   - » Pipette controller
   - » Serological pipettes (2)
   - » Tween 20
   - » Used flow cell
   - » White wash tray

   —A used flow cell might already be present on the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 37.5 ml 4.00–4.99% sodium hypochlorite to the bottle to prepare 1.54 L ~0.12% sodium hypochlorite.
5. Label the bottle **Wash 1 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 1 Solution at room temperature. Use within the day or discard.
8. Add 1.5 L nuclease-free water to a new 2 L bottle.
9. Attach a new serological pipette to the pipette controller.
10. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
11. Label the bottle **Wash 2 Solution**.
12. Cap the bottle and invert several times to mix.
13. Set aside Wash 2 Solution at room temperature.

Initiate a Maintenance Wash

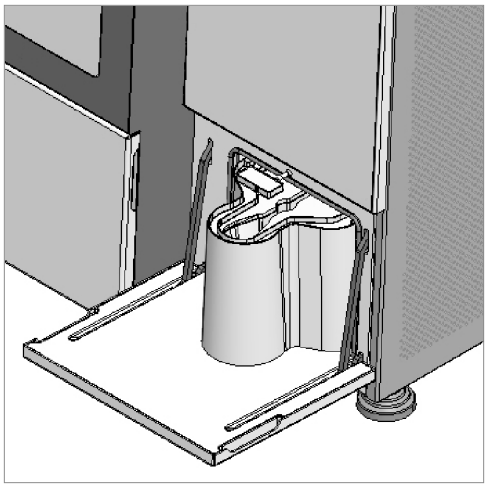
1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a ***used*** flow cell:
   a. Select **Open Nest**.
   b. Place the used flow cell onto the nest and close the lid.
   c. Select **Close Nest**.
3. Select which side to wash:
   - » **Side A**—Set up a maintenance wash on side A.
   - » **Both**—Set up maintenance washes on sides A and B.

» **Side B**—Set up a maintenance wash on side B.

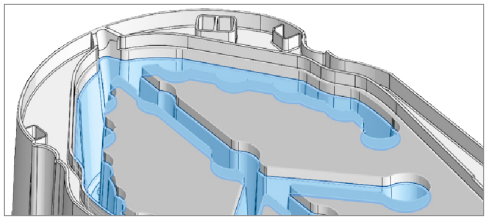
4. Select **Wash**, and then select **Maintenance**.
5. Select **Next** to proceed to the Load Wash 1 screen.

Load Wash 1 Solution

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered gray wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.



5. Add 590 ml freshly prepared Wash 1 Solution to the fill area, filling the wash tray to slightly above the lower fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. Select **Next** to proceed to the Empty Waste screen.

Empty Waste and Run Wash 1

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

CAUTION
Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.

4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:

   a. Position the bottle over the funnel or waste receptacle.

- If you inserted a funnel, align the handle to the inner edge of the funnel.
- If you did not insert a funnel, center the handle over the waste receptacle.

b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.

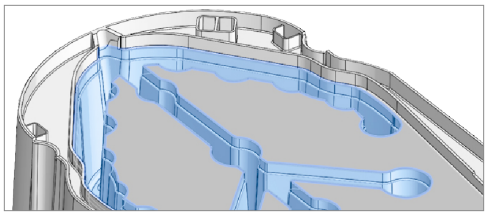
c. If necessary, wipe liquid off the bottle.

7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Wash 1 screen and automatically start the wash, which takes ~34 minutes.
10. During the wash, process the materials removed from the reagent bay:
    - » If you removed a used buffer bottle and cartridge basket, follow the discard instructions in the user guide for the kit.
    - » If you removed a wash tray, follow the guidelines in *Wash Tray Maintenance* on page 28.
11. When the wash is complete, select **Next** to proceed to the Load Wash 2 screen.

Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the gray wash tray from the reagent bay and set aside.

   —Residual liquid in the wash tray is normal.—
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

Run Wash 2

1. Select **Next** to open the Run Wash 2 screen and automatically start the wash, which takes ~52 minutes.
2. When the wash is complete, select **Done** to return to the Home screen.
3. Leave all materials in the instrument.
4. Process the gray wash tray from the first wash per *Wash Tray Maintenance* on page 28.

Perform a Standby Wash

A standby wash takes ~52 minutes and flushes nuclease-free water through the fluidic system, removing any residual Tween 20. When complete, the washed side is idle. Performing a maintenance wash on the idle side ends the idle period and enables sequencing.

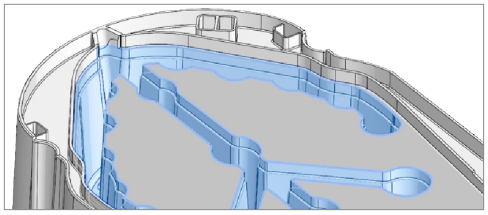
Initiate a Standby Wash

1. Gather the following materials:
   - » Nuclease-free water
   - » Used flow cell
   - » White wash tray

   —A used flow cell might already be present on the instrument.—
2. On the Home screen, select **New Run**.
3. If AVITI OS prompts that the flow cell is missing, load a ***used*** flow cell:
   a. Select **Open Nest**.
   b. Place the used flow cell onto the nest and close the lid.
   c. Select **Close Nest**.
4. Select which side to wash:
   - » **Side A**—Set up a standby wash on side A.
   - » **Both**—Set up standby washes on sides A and B.
   - » **Side B**—Set up a standby wash on side B.
5. Select **Wash**, and then select **Standby**.
6. Select **Next** to proceed to the Load Water screen.

Load Nuclease-Free Water

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door.
8. Select **Next** to proceed to the Empty Waste screen.

Empty Waste and Run the Standby Wash

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

   CAUTION
   Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:

   a. Position the bottle over the funnel or waste receptacle.
      - If you inserted a funnel, align the handle to the inner edge of the funnel.
      - If you did not insert a funnel, center the handle over the waste receptacle.

   b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.

   c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Water screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Remove Tray screen.

Unload the Wash Tray

1. When prompted, open the reagent bay door and remove the wash tray.

   —Residual liquid in the wash tray is normal.—
2. Close the reagent bay door.
3. Select **Done** to proceed to the Home screen.
4. Leave the flow cell in the nest.
5. Process the materials removed from the reagent bay:

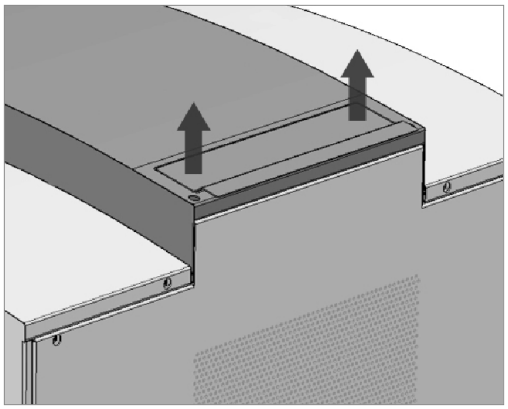
   » If you removed a used buffer bottle and cartridge basket, follow the discard instructions in the user guide for the kit.

   » If you removed a wash tray, follow the guidelines in *Wash Tray Maintenance* on page 28.

Replace the Air Filter

To ensure proper cooling and continuous operation of the system, replace your air filter every 12 months. If your site is located at a high elevation, replace your air filter every 6 months. For more information, see the *AVITI24 System Site Prep Guide (MA-00052)*.

1. If the instrument is performing a run or washing, wait for the run or wash to complete.
2. Select the user menu, and then select **Shut Down**.
3. When prompted, select **Shut Down** again to shut down the instrument computer.
4. Wait for the screen to go blank and a No Signal message to appear.
5. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.
6. Using the flange toward the back of the instrument, lift the air filter tray out of the top.



7. Remove the air filter from the tray and discard.

   —The filter might be loose in the tray, which is normal.—
8. Place the tray on a table or benchtop.
9. With the small arrow on the side of the filter pointing up, place the new air filter into the tray.
10. Lower the tray into the instrument. Use the pins to align the tray to the rails and guide entry.
11. On the IO panel, press the power toggle switch to turn on the instrument.

    —The system initializes and displays the Home screen.—

CHAPTER 4

System Configuration

System Connections

The AVITI24 System uses a combination of network, internet, and storage connections to operate. Each system requires a network connection and at least one storage connection. Cloud storage connections, telemetry, over-the-air software updates, and remote support require an internet connection.

Mode	Network Connection	Internet Connection	Storage Connection
Online	Internet	DHCP or static	Cloud or local
	Local	DHCP or static	Local
Offline	Local	None	Local

System Modes

The system mode determines connection options and settings for exporting log files, password protection, and software updates:

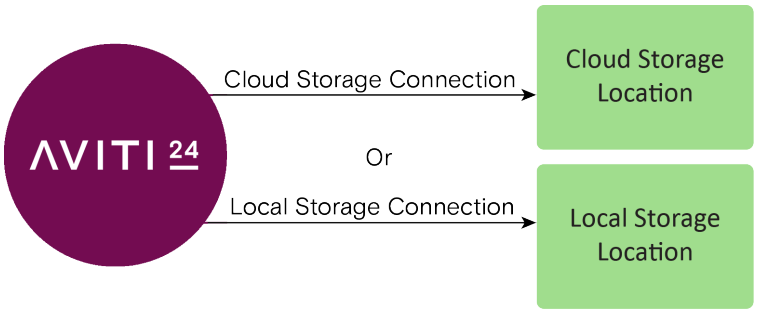
- Online mode connects the system to the internet, which streamlines operations.
- Online local authentication mode operates in online mode but includes local authentication, which avoids network requirements to allowlist Auth0 IP addresses. Only an Element representative can enable this mode.
- Offline mode operates the system without an internet connection. Only an Element representative can enable offline mode.

Storage Connections

A storage connection establishes an off-instrument location that AVITI OS transfers files to. Each run delivers bases files and other run outputs to the default storage location unless you specify a different location during run setup.

AVITI OS supports cloud and local storage connections:

- A cloud storage connection transfers files to a storage location in the cloud.
- A local storage connection transfers files to a storage location on a local network or USB drive.



Storage Connection Requirements

Adding a storage connection requires permissions, network information, and account information that your IT administrator can provide. You must set up cloud storage connections in ElemBio Cloud and fulfill the requirements for the cloud service provider.

For comprehensive storage requirements, see the *AVITI24 System Site Prep Guide (MA-00052)*.

Supported Storage Connections

Cloud storage connections include ElemBio Catalyst, Amazon Web Services (AWS), DNAnexus, and Google Cloud Storage (GCS). For local storage, AVITI OS supports Server Messenger Block (SMB) and USB.

The storage location for a cloud storage connection is a bucket. A connected bucket is available to all systems. Local storage is exclusive to the system.

Cloud Storage

Cloud Storage Connection	Description
ElemBio Catalyst	• Subscription-based service. • Connects the system to an Amazon Simple Storage Service (Amazon S3) bucket that Element creates and operates on your behalf. For more information, see *ElemBio Catalyst* on page 19. • Transfers data using AWS Identity and Access Management (IAM).
AWS	• Connects the system to an Amazon S3 bucket. • Transfers data using secret key authentication through IAM.
DNAnexus	• Connects the system to a DNAnexus project. • Transfers data using an API key for authentication.
GCS	• Connects the system to a Cloud Storage bucket. • Transfers data using secret key authentication through a keyed-hash message authentication code (HMAC).

Local Storage

Local Storage Connection	Description
SMB	• Connects the system to the server running SMB via a path to a folder. • Uses the SMB protocol based on service user authentication to transfer data. • Enables import of a run manifest from an SMB storage location during run setup. • Supports automatic export of log files from offline systems. • Supports Kerberos or NTLMv2 authentication.
USB	• Transfers data and log files to a USB drive connected to the instrument. • Supports automatic and manual export of log files from offline systems. • Supports USB-A 3.0 or newer versions and FAT32 or exFAT formats. • Must store ≥ 1.6 TB of data, which is sufficient for at least two full runs.

Configure General Settings

General settings include the instrument name setting, the on-screen keyboard setting, file output settings for instrument priming and wash runs, air filter time resetting, and read-only settings that control the instrument profile. For offline systems, general settings also include features to export log files. For instructions, see *Exporting Log Files* on page 46.

Name the Instrument

1. On the taskbar, select **Settings**.
2. Select the **General** tab, and then select **Edit**.
3. Enter a preferred name consisting of 1–20 alphanumeric characters, hyphens (-), and underscores (_) to identify the instrument.

   —The default name is the serial number, field-programmable gate array (FPGA) ID, or Unnamed Instrument.—
4. Select **Save** to apply the name.

Configure On-Screen Keyboard

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Select the **Show on-screen keyboard** toggle to enable or disable the on-screen keyboard for text-entry fields.

Configure File Output Settings

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Select the **Save prime output to storage** toggle to enable or disable the output files for instrument priming.
4. Select the **Save wash run output to storage** toggle to enable or disable the output files for a wash run.

   —When you enable the setting, AVITI OS requires you to configure a storage connection before you start a wash.—

Review Read-Only Settings

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Review the following read-only settings. To change a setting, contact Element Technical Support.

Setting	Default	Description
High Elevation	Disabled	Calibrates the system to operate at a high elevation
Offline Mode	Disabled	Prevents an internet connection

Reset the Air Filter Time

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. From the Reset Air Filter Time setting, select **Reset**.
4. When prompted, select **Reset** again to confirm that you have replaced your air filter. The timer will be reset.

Connect to the Network

Network settings connect the system to your network via a Dynamic Host Configuration Protocol (DHCP) or a static IP address. When the system is connected to an Ethernet port, AVITI OS automatically connects to a DHCP server and autopopulates the network settings. Alternatively, you can assign a static IP address and manually configure the network settings.

Select a DHCP Server

1. On the taskbar, select **Settings**.
2. Select the **Network** tab.
3. In the drop-down menu, select **Automatic (DHCP)**.

   —AVITI OS assigns a dynamic IP address and all other network settings.—

Assign a Static IP Address

1. On the taskbar, select **Settings**.
2. Select the **Network** tab.
3. In the drop-down menu, select **Manual**.

   —AVITI OS assigns a unique and permanent IP address.—
4. Select **Edit**, and then configure the following network settings.

Setting	Example	Description
IP Address	11.2.34.178	The destination IP address
Gateway	11.2.34.177	The IP address of the gateway computer that manages network communications
Subnet Mask	11.2.34.176	The subnet mask that separates the IP address into host and network addresses
Name Server IP(s)	ngs-1.yourlab.com	The names of up to four Domain Name System (DNS) servers that provide IP addresses

   —Two additional network settings, Host name and Mandatory Access Control (MAC) address, are read-only.—
5. Select **Save** to apply the settings and connect to the network.

Add Storage Connections

The Storage tab lists storage connections for the system, including available storage for each local storage connection. An Element representative adds the first storage connection at installation. After installation, you can add an unlimited number of additional storage connections.

AVITI OS only lets you add local storage connections. To add a cloud storage connection, access ElemBio Cloud. For more information, see *Storage Connections* in the *Online Help*.

Add an SMB Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Add Storage**, and then select **Local File Server (SMB)** as the storage provider.
4. In the Name field, enter a preferred name for the storage connection.
5. Complete the following fields to configure an SMB network storage location for the SMB storage connection.

Field	Instruction
Host	Enter the host network IP address or fully qualified domain name (FQDN). The Kerberos authentication protocol requires an FQDN. • Example IP address: 1.222.333.44 • Example FQDN: datapc.elembio.com
Port	Enter a port number for the file transfer service or leave blank to accept the default of port 445.
Workgroup/Domain	Enter the name of the workgroup or domain that the user belongs to. The Kerberos authentication protocol requires you to enter the Kerberos realm name. • Example workgroup/domain for Kerberos: elembio.com
Share	Enter the name of the share that makes the directory accessible to SMB.
Path	Enter the path to an ***existing*** folder where you want to output data.
User	Enter the user name for the service user.
Password	Enter the password for the service user.

—All fields except Port and Path are required. Certain server configurations require a work group or domain.—

6. In the Temporary Prefix drop-down menu, select **Disabled** or **Enabled**.

   —The Temporary Prefix setting appends two underscores to the name of a file (e.g., __ExampleFileName.zip) while in transfer to the SMB location. The prefix disappears when the file transfer finishes.—

7. In the Session Security drop-down menu, select a setting for the level of encryption:
   - **High (Recommended)**—AVITI OS requests an encrypted connection with the SMB server. This option is the default setting.
   - **Medium**—The SMB server determines use of an encrypted connection. The server determines if a connection is encrypted or signed.
   - **Low**—AVITI OS disables extended SMB security negotiation (SPNEGO) for wider compatibility with SMB servers. The SMB server determines if a connection is encrypted or signed.
8. In the File Checksums drop-down menu, select **Disabled** or **Enabled**.

   —The File Checksums setting computes the MD5 checksum for each transferred file and lists them in the RunUploaded.json file. You can use this information to verify the integrity of files.—

9. If prompted, select **Confirm** to set the Session Security selection.
10. Select **Save** to add the storage connection.

Add a USB Storage Connection

> For a USB storage connection, the instrument supports USB-A 3.0 or newer versions and the FAT32 or exFAT formats. The USB drive must have > 1.6 TB of available storage space, and the USB name can only use alphanumeric characters, hyphens, and underscores.

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **USB Drive** as the storage provider.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. In the Name field, enter a preferred name for the storage connection.
7. Select **Save** to add the storage connection.

   —AVITI OS makes sure the USB drive is connected to the instrument and has write permission and sufficient storage.—

Disconnect the USB for a Storage Connection

1. Select **More** for the USB storage connection, and then select **Eject**.
2. Detach the USB drive from the instrument.
3. To reuse the USB after disconnecting, reconnect the device to a USB port.

   —The USB name must remain the same for AVITI OS to identify the storage connection.—

Manage Storage Connections

Storage settings manage storage connections and include setting the default storage connection. Unless you reset the default storage connection, the default storage connection is the first cloud location added to the instrument. If a cloud location does not exist, the default storage connection is the first local network location.

You can verify any storage connection, but only local storage connections can be edited and deleted in AVITI OS. If you must edit a cloud storage connection, access ElemBio Cloud. For more information, see *Storage Connections* in the *Online Help*.

For ElemBio Catalyst, storage connections that have been expired less than 14 days appear as expired in the storage connections list and cannot be selected in the run setup. Storage connections that have been expired more than 14 days do not appear in the storage connections list. To renew your ElemBio Catalyst subscription, contact your sales representative.

Verify a Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select **More**, and then select **Verify Storage**.
4. Wait ~20 seconds for a success message to appear, indicating a valid storage connection.

   —AVITI OS indicates that the connection is connected, unverified, or partially verified with a blocked network.—
5. If AVITI OS cannot verify the storage connection, troubleshoot:

   a. Make sure the storage connection is correctly set up.
      - For an AWS storage connection, check the IAM permissions. See the applicable JavaScript Object Notation (JSON) policy template in the *Online Help*.
      - For a GCS storage connection, check the role assigned to the HMAC key.
      - For an SMB storage connection, check the permissions associated with the users.
      - For a USB storage connection, make sure the USB is not ejected, and check that the USB name and type are correct. For USB requirements, see *Local Storage* on page 38.

   b. If the storage connection is correctly set up, contact Element Technical Support.

Set the Default Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select **More**, and then select **Set as Default**.
4. When prompted, select **Set Default**.

Edit a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the local storage connection you want to edit, select **More**, and then select **Edit**.

   —Editing a busy storage connection can affect where run output is stored.—
4. On the Edit Storage Connection screen, edit any of the following fields.

Field	Instruction
Name	Enter a preferred name for the storage connection.
Workgroup/Domain	Enter the name of the workgroup or domain that the user belongs to. The Kerberos authentication protocol requires you to enter the Kerberos realm name. • Example workgroup/domain for Kerberos: elembio.com
User	Enter the user name for the service user.
Password	Enter the password for the service user.
Session Security	Select **High (Recommended)**, **Medium**, or **Low**.

—The Host, Share, Port, Path, Temporary Prefix, and File Checksums fields are read-only. If you must edit these fields, create another storage connection.—

5. Select **Save** to apply the edits and update the storage connection.

Delete a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the local storage connection you want to delete, select **More**, and then select **Delete**.
4. When prompted, select **Delete**.

   —AVITI OS does not allow you to delete a busy storage connection.—

Update the Software

AVITI OS checks for new software versions daily and sends a notification when an update is available. The update runs over-the-air and takes 1-2 hours to complete. Make sure that you initiate the update during instrument downtime to avoid disruptions. If an update exceeds 3 hours and you require support, contact ElemBio Support at support@elembio.com.

For offline systems, Element notifies you of an update and provides the files that are needed for a manual update. Manual updates are only available for systems in offline mode. For instructions, see *Perform a Manual Update* on page 49.

Perform an Over-the-Air Update

1. Make sure that the AVITI24 System is not performing a run or wash.
2. Power cycle the system. For more information, see *Power Cycle the System* on page 29.

   —For AVITI OS versions 3.3.0 or later, if you haven't performed a system power cycle in the previous 7 days, the system prompts you to power cycle before you start the update.—
3. On the taskbar, select **Settings**, and then select **Update Software**.
4. When prompted, select **Update Now** to start the update.

   —The system might restart multiple times during the update process.—
5. After the update completes, power cycle the system when prompted.
6. After the system power cycles, select **Notifications** to view a notification that confirms success.
7. If the update is unsuccessful or takes longer than 3 hours, contact Element Technical Support.

   —AVITI OS reverts to the previous version so you can continue operation.—

Manage an Offline System

For AVITI24 Systems in offline mode, AVITI OS lets you export log files, password-protect the system, and perform manual software updates. To install add-ons, offline systems require a specific procedure that uses a USB with an add-on key downloaded from ElemBio Cloud. These procedures and features are unique to offline mode and help manage offline systems.

Exporting Log Files

Offline systems support the export of log files using two methods:

- **Automatic export**—Configure AVITI OS to automatically export log files to a local storage location every hour for telemetry purposes. For help connecting exported log files to telemetry, contact Element Technical Support.
- **Manual export**—Export log files to a USB drive as needed to provide troubleshooting resources to Element Technical Support. AVITI OS lets you perform a limited log file export or a full export of log files.
  - » **Limited Log Export**—Export the log files for a selected run. Use to support initial troubleshooting of a run.
  - » **Full Log Export**—Export all log files for a system. Use to support in-depth system and run troubleshooting.

By default, automatic export is disabled and AVITI OS does not export any log files. When exporting log files to a USB drive, a solid-state drive (SSD) offers significant time savings compared to a flash drive.

Enable Automatic Export of Log Files

1. If necessary, add a local storage connection to export log files to. For instructions, see *Add Storage Connections* on page 41.
2. On the taskbar, select **Settings**.
3. Select the **General** tab, and then select **Set Up Automatic Export**.
4. In the Storage Connection drop-down menu, select a local storage connection.
5. Select **Save** to enable automatic export.
6. Transfer the exported log files to an internet-accessible location for telemetry.
7. Delete transferred files from the storage location.

   —Each automatic export adds log files to the storage location without replacing or removing existing files.—

Disable Automatic Export of Log Files

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Disable** to stop automatically exporting log files.

Change the Automatic Export Location

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Edit**.
4. In the Storage Connection drop-down menu, select a local storage location to export log files to.
5. Select **Save** to reset the location.

Manually Export Full Log Files

1. Connect a USB drive to a USB port on the side or back of the instrument.

2. On the taskbar, select **Settings**.
3. Select the **General** tab.
4. In the drop-down menu for manual exports, select **Full Log Export**.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. Enter an Export Range using the Start Date, End Date, and time fields.

   —The Export Range cannot exceed 14 days.—
7. [Optional] Select the **All Day** toggle to remove time fields and export all log files for the dates in the Export Range.
8. Select **Export Logs**.

   —AVITI OS exports the log files to the USB drive.—
9. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
10. Detach the USB drive from the instrument.
11. Upload the log files to the location that Element Technical Support provides.

Manually Export Limited Log Files

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **General** tab.
4. In the drop-down menu for manual exports, select **Limited Log Export**.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. In the Export Run drop-down menu, select the run for which you want log files.
   - » Only runs from the last 14 days are available.
   - » If you select an active run, only certain files might be available.
   - » If you attempt to export log files soon after an active run starts, you might receive an error message. Wait until the run progresses further and attempt the export again.
7. Select **Export Logs**.

   —AVITI OS exports the log files to the USB drive.—
8. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
9. Detach the USB drive from the instrument.
10. Upload the log files to the location that Element Technical Support provides.

Manage Passwords

User settings manage passwords for offline systems and online systems with local authentication. Offline systems support setting, changing, resetting, and removing passwords. An online system supports password reset and removal only.

NOTE
Resetting or removing a password requires assistance from Element Technical Support.

Set a Password

1. On the taskbar, select **Settings**.
2. Select the **User** tab.
3. In the Password field, enter a new password.

   —The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—

4. In the Confirm Password field, reenter the new password.
5. Select **Save**.
6. When prompted, select **Yes, Set Password**.

Change the Password

1. On the taskbar, select **Settings**.
2. Select **User**.
3. In the Current Password field, enter the current password.
4. In the Password field, enter a new password.
   —The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
5. In the Confirm Password field, reenter the new password.
6. Select **Save** to apply the new password.

Reset a Lost Password

1. On the login screen, select **Forgot Password**.
2. Select **Generate** to display a password reset token and the instrument serial number.
3. Contact Element Technical Support and provide the token and serial number.
   —Element Technical Support emails you a single-use password reset file.—
4. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
5. Connect the USB drive to a USB port on the side or back of the instrument.
6. Select **Next**.
7. Select **Load Reset File** to upload the password reset file, which removes the password from the system.
8. In the Password field, enter a new password.
   —The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
9. In the Confirm Password field, reenter the new password.
10. Select **Reset Password** to apply the new password and return to the login screen.
11. Sign in to the system using the new password.
12. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
13. Detach the USB drive from the instrument.
14. Discard the password reset file.

Remove the Password

1. On the taskbar, select **Settings**.
2. Select **User**, and then select **Remove Password**.
3. When prompted, select **Yes, Remove Password**.
4. Select **Generate** to display a password reset token and the instrument serial number.
5. Contact Element Technical Support and provide the token and serial number.
   —Element Technical Support emails you a single-use password reset file.—
6. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
7. Connect the USB drive to a USB port on the side or back of the instrument.
8. Select **Next**.

9. Select **Load Reset File** to upload the password file, which removes the password from the system.
10. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
11. Detach the USB drive from the instrument.
12. Discard the password reset file.

Perform a Manual Update

1. Unzip the files that Element provides for the update.
2. Save the files at the root level of an exFAT USB drive with a minimum storage of 12 GB.
3. Connect the USB drive to a USB port on the side or back of the instrument.
4. Make sure the AVITI24 System is not performing a run or wash.
5. Power cycle the system. For more information, see *Power Cycle the System* on page 29.

   —For AVITI OS versions 3.3.0 or later, if you haven't performed a system power cycle in the previous 7 days, the system prompts you to power cycle before you start the update.—
6. On the taskbar, select **Settings**.
7. Under Software Update, in the USB Drive drop-down menu, select the USB drive that contains the update files.
8. When prompted, select **Update Now** to perform the update.

   —The system might restart multiple times as it updates.—
9. After the update completes, power cycle the system when prompted.
10. After the system power cycles, select **Notifications** to view a notification that confirms a successful update.
11. If the update is unsuccessful or takes longer than 3 hours, contact Element Technical Support.

    —AVITI OS reverts to the previous version so you can continue using the system.—
12. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
13. Detach the USB drive from the instrument.

Install Add-Ons on an Offline System

> Add-ons on offline AVITI24 Systems require an installation procedure using a USB with a key downloaded from ElemBio Cloud. The add-on key file must be located at the root level of the USB. For instructions to download the add-on key, see *Add-Ons* in the *Online Help*.

1. Use a USB port on the side or back of the instrument to connect the USB drive with the add-on key file downloaded from ElemBio Cloud.
2. On the taskbar, select **Settings**.
3. Select the **Add-Ons** tab.
4. In the drop-down menu, select the USB Drive.
5. Select **Upload**.

   —AVITI OS uploads the key file, which installs the add-ons. The Add-Ons tab displays the add-ons and expiration dates.—

CHAPTER 5

Troubleshooting

General Troubleshooting

Error messages communicate hardware or software problems and describe both the problem and resolution. General troubleshooting resolves other problems that can occur during system initialization, run setup, and run progression. If a problem persists, contact Element Technical Support.

A power cycle resolves many common problems. For instructions, see *Power Cycle the System* on page 29.

Problem	Resolution
After turning on the instrument, the monitor does not display AVITI OS.	Power cycle the system.
The initialization sequence does not complete, and the loading screen persists.	
The software, instrument, keyboard, or mouse stop operating.	
The reagent or waste bay contains liquid.	See *Clean Spills and Leaks* on page 56.
Liquid is spilling from the front or bottom of the instrument.	
The nest is wet.	
AVITI OS detects a full waste bottle, but the bottle is empty.	Reload the waste bottle and make sure the waste bay is unobstructed.
The reagent bay contains condensation.	Dry the inside of the reagent bay with a clean, dry microfiber cloth. Clean to the back of the bay, avoiding sensors and cables.
The instrument does not accept a USB device.	Confirm that the USB device meets the following requirements: • Contains > 1.6 TB available space. • Uses USB-A 3.0 or a newer version. • Uses the FAT32 or exFAT format. • Uses a name with alphanumeric characters, hyphens, and underscores only.
AVITI OS does not proceed with run setup despite sufficient storage space on the instrument.	Set up a storage connection to use with the run. A storage connection is required for a run, and on-instrument storage is reserved as a backup in case of network disruption.
A run continues after you stop it.	Wait for the run to stop. AVITI OS waits for a safe point to stop the run, which can take several minutes to ~2 hours depending on the run stage.
Network connection is lost in the middle of a run.	Wait for the AVITI24 System to reconnect to the network. Sequencing and cytoprofiling chemistry are not impacted by network disruptions, and the run progresses as expected. After the system reconnects, data transfer resumes. The system has enough local disk storage for two sequencing runs.

Problem	Resolution
Connection to storage location is lost, and the instrument cannot reconnect.	Confirm with your IT department that all necessary ports and URLs in the *AVITI24 System Site Prep Guide (MA-00052)* are allowlisted.
The run folder is missing data.	Make sure the user interface indicates that the system is uploading and wait for the upload to complete. • A slow connection delays data transfer. • Data transfer failure prompts a notification.

Cancel Runs and Washes

AVITI OS displays the following buttons for canceling runs and washes:

- **Discard**—Cancels run or wash setup. The button appears when you can discard setup without compromising consumables.
- **Stop**—Appears on the Home screen and cancels an active run. The button is always enabled so you can free the instrument when run parameters are incorrect, data quality is poor, or a hardware problem occurs.

Discard Run Setup

1. On any run setup screen before priming, select **Discard**.
2. When prompted to confirm the discard, select an option:
   - » **Unlock Door A** or **Unlock Door B**—Discard the run and save the cartridge.
   - » **Discard Setup**—Discard the run, delete the run, and return to the Home screen without saving the cartridge.
3. If you unlocked the door, proceed with the remaining steps.
4. Open the reagent bay door and remove the cartridge.
5. Place the cartridge on ice or refrigerate at 2°C to 8°C.
6. Place a clean, uncovered white wash tray onto the open reagent bay door.
7. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
8. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.
9. Slide the wash tray all the way into the reagent bay until it stops.
10. Close the reagent bay door.
    —AVITI OS deletes the run setup and returns to the Home screen.—
11. Set up a new run and use the cartridge within ***4 hours***.

Discard Wash Setup

1. On any wash setup screen, select **Discard**.
2. When prompted to confirm the discard, select **Discard Setup**.
   —AVITI OS deletes the wash setup and returns to the Home screen.—

Stop an Active Run

Stopping an active run is a two-part process: stop the run, then perform a ~60-minute recovery wash to remove residual library from the fluidic system.

CAUTION
Stopping a run is ***final***. You cannot resume a stopped run or reuse any of the consumables.

Stop the Run

1. On the applicable side of the Home or Run Details screen, select **Stop**.
2. When prompted, select **Yes, Stop Run**.
   —AVITI OS finishes the current step, terminates the run, and returns to the Home screen.—
3. Proceed to *Prepare Wash 2 Solution* on page 54 and complete the recovery wash.

Prepare Wash 2 Solution

1. Gather the following materials:
   - » 2 L bottle
   - » Nuclease-free water
   - » Pipette controller
   - » Serological pipette
   - » Tween 20
   - » Used flow cell
   - » White wash tray

   —A used flow cell might already be present on the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
5. Label the bottle **Wash 2 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 2 Solution at room temperature.

Initiate a Recovery Wash

1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a ***used*** flow cell:
   a. Select **Open Nest**.
   b. Place the used flow cell onto the nest and close the lid.
   c. Select **Close Nest**.
3. Select which side to wash:
   - » **Side A**—Set up a recovery wash on side A.
   - » **Both**—Set up recovery washes on sides A and B.
   - » **Side B**—Set up a recovery wash on side B.
4. Select **Wash**, and then select **Recovery**.
5. Select **Next** to proceed to the Load Wash 2 screen.

Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the buffer bottle and cartridge basket from the reagent bay. Set aside both materials.
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.
6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door, and select **Next** to proceed to the Empty Waste screen.
8. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

Empty Waste and Run Wash 2

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

   CAUTION
   Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:

   a. Position the bottle over the funnel or waste receptacle.
      - If you inserted a funnel, align the handle to the inner edge of the funnel.
      - If you did not insert a funnel, center the handle over the waste receptacle.

   b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.

   c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Wash 2 screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Home screen.
11. Discard the cartridge and buffer bottle and wash the basket. See the discard instructions in the user guide for your sequencing or cytoprofiling kit.

Clean Spills and Leaks

Clean the nest, waste bay, or reagent bay to recover from a leak or spill observed when setting up a run or wash. A leak or spill that occurs in the waste bay during a run causes an error and requires cleaning and contacting Element Technical Support.

If the bottom of the instrument is leaking or liquid is spilling from the front of the instrument: shut down and unplug the instrument if doing so is safe and contact Element Technical Support.

Clean the Nest

1. Dampen a microfiber cloth with isopropyl alcohol.
2. Wipe the nest with the damp microfiber cloth and allow to dry.
3. If necessary, use a polyurethane foam-tip swab to clean additional areas around the nest.
4. Resume run or wash setup.

Clean the Reagent Bay

1. Keep the reagent bay door open.
2. Remove any materials from the reagent bay and set aside.
3. Wipe the interior of the reagent bay with a damp microfiber cloth, cleaning to the back of the bay while avoiding sensors and cables.
4. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
5. Resume run or wash setup.

Clean the Waste Bay

1. Keep the waste bay door open. If the leak occurs during a run, open the door:
    a. Wait for any runs or washes on the unaffected side to finish.
    b. On the taskbar, select **Notifications**.
    c. On the applicable error, select **Unlock Waste Module Door**.
    d. Open the waste bay door.
2. Unscrew the transport cap from the cap holder on the affected side.
3. Remove the waste bottle from the waste bay and close the transport cap.

    CAUTION
    Waste droplets might be on the exterior of the waste bottle.
4. Inspect the waste bottle for cracks, holes, and other defects.
5. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
6. Open the transport cap and the vent cap.
7. Support the waste bottle with both hands and empty the waste:
    a. Position the bottle over the funnel or waste receptacle.
        - If you inserted a funnel, align the handle to the inner edge of the funnel.
        - If you did not insert a funnel, center the handle over the waste receptacle.
    b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.

c. If necessary, wipe liquid off the bottle.

8. Close the vent cap, leave the transport cap open, and set aside the waste bottle.
9. Wipe the interior of the waste bay with a damp microfiber cloth.
10. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
11. Return the waste bottle to the waste bay.
    - » If the bottle is defective and you have a spare, load the spare.
    - » If the bottle is defective and you do not have a spare, load the defective bottle. Do not use the affected side until the defective waste bottle is replaced.

    —A run or wash on either side requires the presence of both bottles.—
12. Screw the transport cap onto the cap holder and close the waste bay door.
13. Resume run or wash setup. If necessary, set up a new run with new consumables and clean accessories.

CHAPTER 6

Safety and Compliance

General Safety

Review this chapter before operating or maintaining the AVITI24 System to ensure safe, correct usage. The procedures described in this guide are tested and optimized, so any deviation can compromise results, cause personal injury, or damage the instrument. All personnel operating the instrument must be trained in correct operation and safety.

The *AVITI24 System Site Prep Guide (MA-00052)* provides delivery information and installation requirements, including instrument specifications, power specifications, and environmental conditions. A field service engineer (FSE) installs the AVITI24 System.

WARNING
Do not attempt to move the instrument, which can result in injury. Only trained Element personnel are qualified to safely move the instrument.

Safety Labels

The following table lists the safety labels affixed to the instrument. The labels identify potential hazards associated with installation, service, and operation. Follow the procedures in this guide as described to avoid interactions that expose you to these hazards.

WARNING
This product can expose you to chemicals including formaldehyde, which is known to the State of California to cause cancer, and methanol, which is known to the State of California to cause birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov.

Potential Hazard	Label	Description
Class 4 Laser		The instrument is a Class 1 laser product that contains a Class 4 laser. See *Laser Safety* on page 60.
Heat hazard		The nest has a hot surface and exposure can cause burns.

Laser Safety

The AVITI24 System is certified as a Class 1 laser product per the US Federal Product Performance Standard for Laser Products requirements described in 21 CFR Subchapter J. The exception to these requirements is the deviations described in FDA Laser Notice #56. The product is classified per IEC/EN 60825-1:2014.

WARNING
Adjusting or performing procedures other than those described in this guide or other Element guides can result in hazardous radiation exposure.

Class 4 Laser

The instrument is a Class 1 laser product that contains a Class 4 laser. The Class 4 laser produces Class 4 levels of visible laser radiation, which can be hazardous to eyes and skin. Protective shells and safety interlocks prevent exposure or access to laser radiation levels that exceed Class 1 during operation, maintenance, or normal service.

The following figure depicts the label that identifies noninterlocked portions of the shells that prevent access to laser radiation. Additionally, the nest bay and both reagent bays contain barcode scanners that emit Class 1 levels of laser radiation.

Label identifying noninterlocked locations

Operating Conditions

Do not operate an AVITI24 System with bypassed interlocks, damaged shells, or any portion of the shells removed. These conditions make Class 4 levels of laser radiation possible and risk exposure to direct or reflected laser light.

Only Element service personnel, Element-authorized agents, or Element-trained personnel can perform services that require internal interlock bypass or removal of portions of the shells. If you are present during service, take the proper safety precautions to mitigate the risk of direct and reflected laser light.

Product Compliance

The AVITI24 System meets the Canadian, EU, South Korean, UK, and US requirements for safety and electromagnetic compatibility (EMC). The system has been tested to and complies with the standards in the following sections.

US and Canadian Safety and EMC Standards

The AVITI24 System is certified to the following safety standards:

- IEC 60825-1, safety of laser products
- IEC 61010-1, general safety requirements for electrical equipment for measurement, control, and laboratory use
- IEC 61010-2-010, particular requirements for laboratory equipment for the heating of materials
- IEC 61010-2-081, particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes

The system also has been tested to and complies with the following EMC requirements:

- FCC 47 CFR Part 15, title 47: telecommunication; part 15 – radio frequency (RF) devices
- ICES-003, information technology equipment (including digital apparatus)

FCC Compliance Statement

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

EU Safety and EMC Standards

The AVITI24 System has been tested to and complies with the following safety standards:

- Low Voltage Directive 2014/35/EU
  - » EN 61010-1, general safety requirements for electrical equipment for measurement, control and laboratory use
  - » EN 61010-2-010, particular requirements for laboratory equipment for the heating of materials
  - » EN 61010-2-081, particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes
  - » EN 60825-1, safety of laser products

The system has been tested to and complies with the following EMC standards:

- EMC Directive 2014/30/EU, EMC requirements
  - » EN 61326-1, general EMC requirements for electrical equipment for measurement, control and laboratory use

The system also complies with the Restriction of Hazardous Substances (RoHS) Directive (2011/65/EU) as amended by the Directive (EU) 2015/863. The directives restrict the use of certain hazardous substances in electrical and electronic equipment.

UK Safety and EMC Standards

The AVITI24 System has been tested to and complies with the following safety standards:

- S.I. 2016 No. 1101, general safety regulations

- » BS EN 61010-1, general safety requirements for electrical equipment for measurement, control and laboratory use
- » BS EN IEC 61010-2-010, particular requirements for laboratory equipment for the heating of materials
- » BS EN IEC 61010-2-081, particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
- » BS EN 60825-1, safety of laser products

The system also has been tested to and complies with the following EMC standards:

- S.I. 2016 No.1091, EMC requirements
  - » BS EN IEC 61326-1, general EMC requirements for electrical equipment for measurement, control and laboratory use

South Korea EMC and Regulatory Compliance

The AVITI24 System has been tested to and complies with the following EMC standards:

- KS C 9610-6-2, electromagnetic immunity standards for equipment used in industrial environments
- KS C 9610-6-4, electromagnetic emission standards in industrial environments

Additional regulatory information to comply with South Korean regulations (in Korean and English):

- 이 기기는 업무용 환경에서 사용할 목적으로 적합성평가를 받은 기기로서 가정용 환경에서 사용하는 경우 전파간섭의 우려가 있습니다.
- "This equipment has been evaluated for its suitability for use in a business environment. When used in a residential environment, there is a concern of radio interference."

Regulatory Markings

The following markings indicate that the instrument complies with conformity requirements, including EMC and safety requirements, for Australia, Canada, the EU, South Korea, the UK, and the US.

Symbol	Description
C N US 61010-1	Nemko Electrical Safety Certification Mark for US and Canada
	Australia Regulatory Compliance Mark
CE	European Conformity (CE) Marking
UK CA	UK Conformity Assessed Marking
R-R-EB6-710-00975	South Korea Conformity Assessment Marking

Index

Document History

Revision	Description of Change
April 2025 Document # MA-00051 Rev. D	• Updated the General tab description in Settings. • Updated note in Run Monitoring to include content for cytoprofiling. • Added links to sequencing and cytoprofiling metrics and charts. • Updated the maintenance wash requirement. • Added ElemBio Catalyst to Supported Storage Connections. • Added ElemBio Catalyst to Add-Ons. • Added Polony Density to Add-Ons. • Updated the estimated completion time for software updates. • Added recommendation to perform software updates during downtime. • Updated title of Perform a Remote Update to Perform an Over-the-Air update and added power cycle steps. • Updated USB drive specifications for manual software updates. • Added power cycle steps for manual software updates. • Added replacement intervals for air filters. • Updated Nemko symbol in Regulatory Markings.
January 2025 Document # MA-00051 Rev. C	• Removed description of cartridge shipping configurations, such as shipping locks or shipping cover.
December 2024 Document # MA-00051 Rev. B	• Added list of additional documentation for workflow instructions. • Added Trinity to the list of kits that are not compatible with individually addressable lanes. • Specified that Cloudbreak cartridges include shipping locks and Trinity cartridges include a thermoform cover. • Updated images of Teton flow cell aligner and sealer to the latest configuration. • Removed the lid from wash tray description.
October 2024 Document # MA-00051 Rev. A	• Initial release

Technical Support

Visit the Documentation page on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

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EXHIBIT 21

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Sequencing by avidity enables high accuracy with low reagent consumption

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We present avidity sequencing, a sequencing chemistry that separately optimizes the processes of stepping along a DNA template and that of identifying each nucleotide within the template. Nucleotide identification uses multivalent nucleotide ligands on dye-labeled cores to form polymerase–polymer–nucleotide complexes bound to clonal copies of DNA targets. These polymer–nucleotide substrates, termed avidites, decrease the required concentration of reporting nucleotides from micromolar to nanomolar and yield negligible dissociation rates. Avidity sequencing achieves high accuracy, with 96.2% and 85.4% of base calls having an average of one error per 1,000 and 10,000 base pairs, respectively. We show that the average error rate of avidity sequencing remained stable following a long homopolymer.

Avidity sequencing chemistry enables a diversity of applications that include single-cell RNA sequencing (RNA-seq) and whole-human-genome sequencing. For the human sample HG002, avidity sequencing reached a single-nucleotide polymorphism (SNP) F1 score of 0.9958 and small-indel F1 score of 0.9954.

Over the past 15 years, highly parallel sequencing methods have enabled a broad set of applications[1–8]. Multiple technologies have been introduced during this time, each having various strengths and limitations[9]. The technologies vary by accuracy, read length, run time and cost. The most widely used method uses highly parallel and accurate short-read sequencing, described in ref. 10 and termed sequencing by synthesis (SBS).

The SBS methodology sequences DNA by controlled (that is, one at a time) incorporation of modified nucleotides[11]. The modifications consist of a 3′ blocking group and a dye label[12,13]. The blocking group ensures that only a single nucleotide is incorporated, and the dye label enables identification of each nucleotide following an imaging step. The blocking group and label are subsequently removed, completing the sequencing cycle. The cycle is repeated with the incorporation of the next blocked and labeled nucleotide. Incorporation of the modified nucleotide meets two objectives: to advance the polymerase along the DNA template and to differentially label the incorporated nucleotide for base identification. Although combination of the two processes is efficient, it prevents independent optimization of the processes. High-yielding and rapid incorporation requires micromolar concentrations of nucleotides to drive the polymerizing reaction[14–18]. The alternative, of allowing longer incorporation times, results in longer cycle times that have an additive effect over 300 cycles of stepwise sequencing.

We present a different sequencing chemistry, termed avidity sequencing, that separates and independently optimizes the controlled incorporation and nucleotide identification steps to achieve increased base-calling accuracy relative to SBS while reducing the concentration of key reagents to nanomolar scale. To advance this approach, we first had to overcome the technical challenge of signal persistence. For example, a potential strategy for separation of the steps described above could be to first incorporate a 3′ blocked but unlabeled nucleotide and then to bind a complementary labeled nucleotide to the subsequent base in the template for base identification. This approach is problematic because the dissociation rate for single nucleotides from a polymerase–template complex is large, and the polymerase–nucleotide complex does not remain stable throughout imaging unless prohibitively high concentrations of nucleotides are present in the bulk solution. To overcome this challenge, we used avidity.

Avidity refers to the accumulated strength of multiple affinities of individual noncovalent binding interactions, which can be achieved when multivalent ligands tethered in close proximity simultaneously bind to their targets[19]. Coincident binding increases ligand affinity and residence time[20]. As an example of the potential impact of avidity on both affinity and decreased dissociation rate, Zhang et al.[21] demonstrated that, by changing a monomeric to a pentameric nanobody, it is possible to decrease dissociation rates by three to four orders of magnitude. Our approach was to use avidity for nucleotide detection within the sequencing chemistry (Fig. 1). We demonstrate here that avidity sequencing achieves accuracy, surpassing an average of one error per 10,000 base pairs (bp) ($Q40$), and enables a diversity of applications that include single-cell RNA-seq and whole-human-genome

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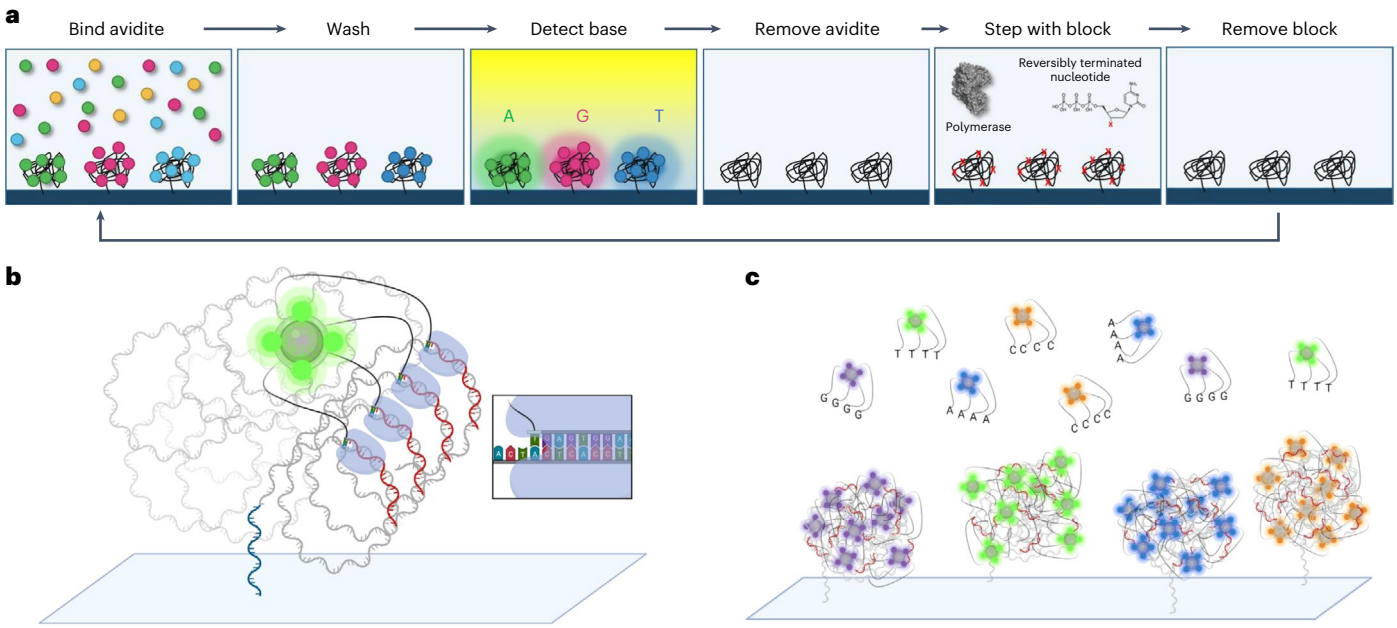

Fig. 1 | Avidity sequencing workflow and scheme. a, Sequencing by avidity. A reagent containing multivalent avidite substrates and an engineered polymerase are combined with DNA polonies inside a flowcell. The engineered polymerase binds to the free 3′ ends of the primer-template of a polony and selects the correct cognate avidite via base-pairing discrimination. The multivalent avidite interacts with multiple polymerases on one polony to create avidity binding that reduces the effective K_d of the avidite substrates 100-fold compared with a monovalent dye-labeled nucleotide, allowing productive binding of nanomolar concentrations. Multiple polymerase-mediated binding events per avidite ensure a long signal persistence time. Imaging of fluorescent, bound avidites enables base classification. Following detection, avidites are removed from the polonies. Extension by one base using an engineered polymerase incorporates an unlabeled, blocked nucleotide. A terminal 3′ hydroxyl is regenerated on the DNA strand, allowing repetition of the cycle. **b**, Rendering of a single avidite bound to a DNA polony via polymerase-mediated selection. The initial surface primer used for library hybridization and extension during polony formation is shown in blue. Sequencing primers (red) are shown annealed to the single-strand DNA polony (gray). Each arm of the avidite (black) connects the avidite core containing multiple fluorophores (green) to a nucleotide substrate. The polymerase bound to the sequencing primer selects the correct nucleotide to base pair with the templating base (inset). The result is multiple base-mediated anchor points noncovalently attaching the avidite to the DNA polony. **c**, Rendering of multiple DNA polonies with template-specific avidites bound during the binding step of the cycle (polymerase not shown for simplicity). Many avidites bind to each DNA polony generating a fluorescent signal during detection. Multiple long, flexible polymer linkers connect the core to the nucleotide substrates.

sequencing. We also demonstrate an improved ability of this chemistry to sequence through homopolymer sequences.

Results

Before sequencing, DNA fragments of interest were circularized and captured on the surface of a flowcell. Clonal copies of DNA fragments were then created through rolling circle amplification, generating approximately 1 billion concatemers on the flowcell surface[22–25]. The resulting concatemers, referred to as polonies using the original term coined by Church and collaborators[26], were used as the DNA substrate for sequencing. In contrast to the DNA nanoballs developed by Complete Genomics, polonies are amplified on-instrument following library hybridization to the flowcell[27]. This approach simplifies user workflow and eliminates the possibility that DNA fragments may interact in solution during the amplification process. We then constructed the avidite: a dye-labeled polymer with multiple, identical nucleotides attached. In the presence of a polymerase, the avidite was able to bind multiple complementary nucleotides specifically in concatemer copies of a DNA fragment within a polony. A polymerase and a mixture of four avidites, each corresponding to a particular label and nucleotide, were applied to the flowcell and used for base discrimination. The avidite was not incorporated, but provided a stable complex while enabling removal under specifically formulated wash conditions. Removal of the avidite left no modifications in the synthesized strand. The avidites decreased the required concentration of reporting nucleotides by 100-fold relative to single-nucleotide binding, yielded negligible dissociation rates and obviated the need to have nucleotides present in the bulk solution. A low avidite concentration leads to reduced use of fluorophores relative to the strategy of using high-concentrations of dye-labeled nucleotides. The advent of the avidite enabled us to separate the process of stepping along the DNA template from the process of identifying each nucleotide, and to optimize each for quality and reagent consumption. Figure 1a shows a complete cycle of avidity sequencing, Fig. 1b depicts a single avidite interacting with multiple DNA copies within a polony and Fig. 1c shows many avidites specifically bound to several polonies on the surface. Additional detail on the structure of one version of an avidite is provided in Extended Data Fig. 1.

Avidity sequencing overcomes the kinetic challenges of generating a signal by incorporation of a dye-labeled monovalent nucleotide. In bulk solution, incorporation of a dye-labeled nucleotide is limited by a specificity constant (k_{cat}/K_m) that governs the observed rate of productive nucleotide binding and incorporation[28]. A specificity constant of $0.54 \pm 0.22\ \mu M^{-1}\ s^{-1}$ for monovalent dye-labeled nucleotides using an engineered polymerase was observed resulting from a maximum rate of incorporation (k_{pol}) of $0.86 \pm 0.14\ s^{-1}$ and an apparent dissociation constant K_d ($K_{d,app}$) of $1.6 \pm 0.6\ \mu M$ (Fig. 2a). This apparent K_d reflects the K_m of a kinetic system not in equilibrium rather than the true K_d of the nucleotide substrate[29]. To achieve complete product turnover, this high apparent K_d can be overcome either by using increased concentrations of fluorescent nucleotide substrate or allowing longer incorporation time for completion of the reaction. Both paths used to overcome this substrate limitation have the undesirable consequence of either high cost or long cycle time. Together, the use of avidity substrates and DNA polonies containing many copies of substrate DNA in close proximity overcomes the limitations of incorporating a monovalent dye-labeled nucleotide.

Using binding of the four labeled avidites for base identification established a binding equilibrium that reached saturation based on

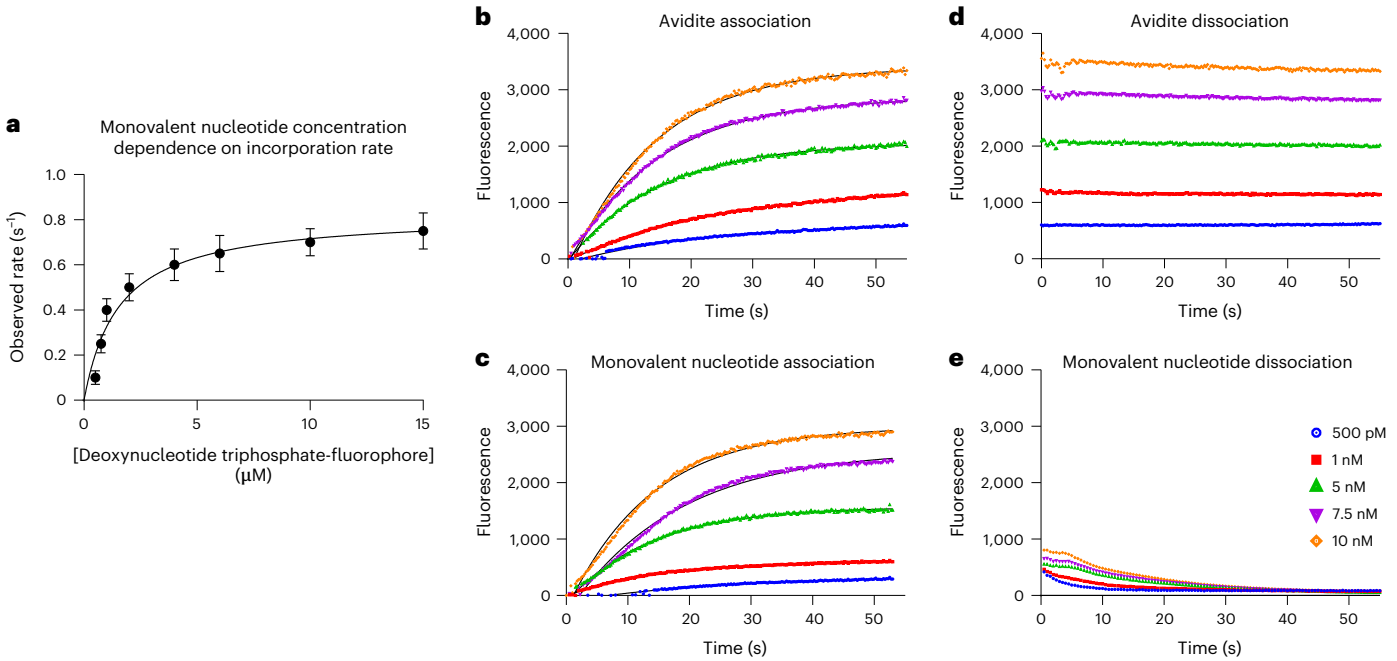

Fig. 2 | Nucleotide and avidite binding kinetics. a, Monovalent fluorophore-labeled nucleotide concentration dependence of the observed rate of incorporation. Time series were performed at each concentration and fit to a single exponential equation to derive a rate. Observed rates were plotted as a function of concentration and fit to a hyperbolic equation, deriving a value of $k_{pol} = 0.86 \pm 0.14\ s^{-1}$ and $K_{d,app} = 1.6 \pm 0.6\ \mu M$. **b**,**c**, Real-time association kinetics of signal generation resulting from reacting multivalent avidite substrates (**b**) and monovalent nucleotides (**c**) with DNA polonies. **d**,**e**, Real-time measurement of signal decay following flow cell washing for imaging of multivalent avidite substrates (**d**) and monovalent nucleotides (**e**).

substrate concentration within 30 s to generate signal, rather than relying on catalysis. The binding kinetics of this interaction were monitored using real-time data collection to observe avidites binding to polonies with an association rate ($k_{on,avidite}$) of $271 \pm 82\ nM^{-1}\ s^{-1}$ (Fig. 2b). This observed association occurred within the limit of error of a single fluorescently labeled monovalent nucleotide (Fig. 2c). Major differences were observed in the dissociation kinetics of avidite substrates versus monovalent nucleotides. Avidite substrates bound to the DNA polonies tightly with no measurable dissociation over the >1-min timescale needed for imaging and base calling (Fig. 2d). This is in sharp contrast to fluorescently labeled monovalent nucleotides, which dissociated rapidly during the wash step following binding and then continued to dissociate during imaging (Fig. 2e). The negligible dissociation rate resulted in decreased K_d of more than two orders of magnitude for avidites compared with monovalent nucleotides. With near-zero avidite dissociation rates, a persistent signal was achieved without the presence of free avidites in bulk solution, eliminating background. Without avidity, dissociation kinetics with monovalent nucleotides showed a fourfold signal decrease at the beginning of imaging due to rapid dissociation, as a result of disruption of the binding equilibrium during reagent exchange (Fig. 2e).

Sequencing instrumentation

Avidity sequencing was performed on the AVITI commercial sequencing system. Briefly, the instrument is a four-color optical system with two excitation lines of approximately 532 and 635 nm. The four-color system is created using an objective lens, multiple tube lenses and multiple cameras for simultaneous imaging of four spectrally separated colors. The detection channels for emission are centered at approximately 553, 596, 668 and 716 nm, respectively. Reagents are delivered using a selector valve and syringe pump to perform reagent cycling. The instrument contains two fluidics modules and a shared imaging module, enabling parallel utilization of two flowcells. Subsequent to image collection, data were streamed through an onboard processing unit that performs image registration, intensity extraction and correction, base calling and quality score assignment (Methods).

Accuracy of avidity sequencing

To evaluate the accuracy of avidity sequencing, 20 sequencing runs were performed using a well-characterized human genome. Sequencing data were used to train quality tables according to the methods of Ewing et al.[30], but with modified predictors. Quality tables were then applied to independent sequencing runs. Figure 3 shows the data quality obtained in a representative run not used for training. Quality scores were well calibrated across the entire range, meaning that predicted quality matched observed quality as determined by alignment to a known reference. Combined over reads 1 and 2, 96.2% of base calls were >*Q*30 (an average of one error per 1,000 bp) and 85.4% >*Q*40, with a maximum of *Q*44, or approximately one error in 25,000 bases. For comparison, a publicly available PCR-free NextSeq 2000 dataset was downloaded from the Illumina public demo set repository (https://basespace.illumina.com/datacentral) and a publicly available NovaSeq 600 dataset (https://console.cloud.google.com/storage/browser/brain-genomics-public/research/sequencing/fastq). The NextSeq 2000 and NovaSeq 6000 datasets had 90.1% and 92.7% of data >*Q*30, respectively, and none of the base calls exceeded *Q*40.

To obtain an additional measure of accuracy, we used the same datasets to compute the percentage of *k*-mers ($k = 1, 2, 3$) containing at least one mismatch after alignment to a well-characterized reference. Known SNP sites were masked before the comparison. When compared with NextSeq 2000 and NovaSeq 6000, we found that AVITI had the highest accuracy across four out of four 1-mers, 16 out of 16 2-mers and 58 out of 64 3-mers (Extended Data Fig. 2).

Homopolymer sequencing

Sequencing through long homopolymers has posed challenges for multiple sequencing technologies[31,32]. Although SBS improves homopolymer sequencing relative to flow-based technologies,

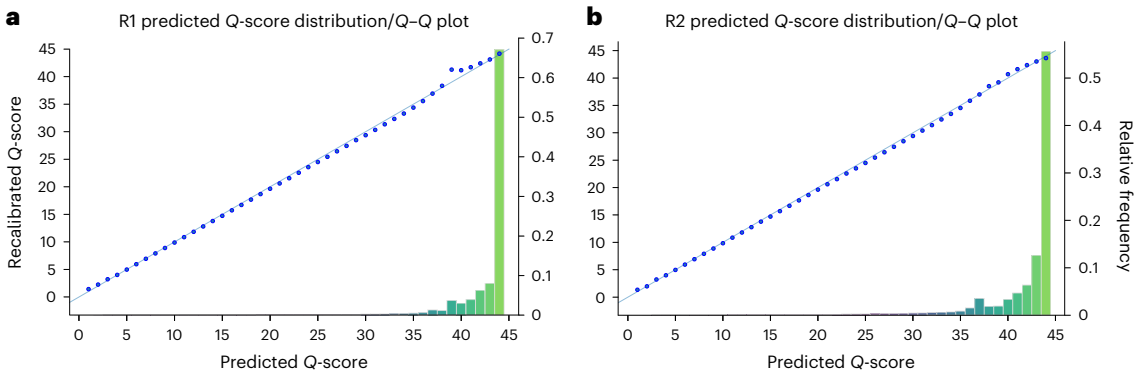

Fig. 3 | Predicted and observed quality scores for a 2 × 150-bp sequencing run of human genome HG002. a, Read 1 (R1). **b**, Read 2 (R2). Points on the diagonal indicate that predicted scores match observed scores. The histograms show that the majority of the data points are >*Q*40.

the error rates of reads that pass through long homopolymer regions increase substantially[33]. Correction algorithms have been proposed to circumvent the inherent challenges with base-calling post-homopolymer repeats[34], but the exact cause has not been fully established in the literature. In contrast to SBS, avidity sequencing leverages rolling circle amplification, polymerases evolved to accommodate the avidite complex formation and a separate polymerase evolved for efficient incorporation of unlabeled and 3′ blocked nucleotides. We evaluated the impact of these differences on sequencing through long homopolymers. Specifically, homopolymers of length 12 or more nucleotides were used to assess the accuracy of reads before and after homopolymer regions. Figure 4 shows the results comparing avidity sequencing with SBS, averaged across the ~700,000 homopolymer loci of length 12 or more. Average error rate of avidity sequencing remained stable following a long homopolymer (controlling for the fact that post-homopolymer stretch occurs in later cycles of a read). By contrast, the error rate of SBS reads increased by more than a factor of five following homopolymer stretches. Extended Data Fig. 3 shows the histogram of pairwise error rate differences between avidity sequencing and SBS for all long homopolymer loci. The avidity sequencing error rate outperformed SBS in >97% of cases and the magnitude of difference is correlated with homopolymer length (Fig. 5). Extended Data Fig. 4 shows representative loci from the 95th, 50th and fifth percentiles of the histogram.

Single-cell RNA-seq

To demonstrate sequencing performance across common applications, single-cell RNA expression libraries were prepared and sequenced. Two libraries from a reference standard consisting of human peripheral blood mononuclear cells were generated using the 10X Chromium instrument. The two libraries contain RNA from roughly 10,000 and 1,000 cells, respectively. Following circularization, the libraries were sequenced to generate paired-end reads with read lengths of 28 and 90 for reads 1 and 2, respectively, as recommended by the vendor. The analysis was done using CellRanger (https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/installation). Because this reference standard is used by 10X Genomics to evaluate sequencing performance, a set of metrics and guidelines to assess sequencing results is provided along with the biological material. Extended Data Table 1 shows each metric, the guideline values from 10X Genomics and the performance of each sequenced library. All metrics were within the guide ranges, and metrics pertaining to sequencing quality exceeded the thresholds provided.

Whole-human-genome sequencing

Another common application is human-whole-genome sequencing. This application challenges sequencer accuracy to a greater extent than measurement of gene expression because the latter requires only accurate alignment while the former depends on nucleotide accuracy to resolve variant calls. To demonstrate performance for this application, the well-characterized human sample HG002 was prepared for sequencing using a Covaris shearing and PCR-free library preparation method and sequenced with 2 × 150-bp reads. The run generated 1.02 billion passing filter paired-end reads with a duplicate rate of 0.58% (0.11% classified as optical duplicates by Picard (https://broadinstitute.github.io/picard/)). To underscore the impact of low duplicates, we compared the number of input reads with genomic coverage (Extended Data Fig. 5).

A FASTQ file with the base calls and quality scores was downsampled to 35-fold coverage and used as an input into the DNAScope analysis pipeline from Sentieon. SNP and indel calls achieved F1 scores of 0.995 and 0.996, respectively. Extended Data Table 2 shows variant-calling performance for SNPs and small indels on the GIAB-HC regions. Sensitivity, precision and F1 scores are shown. The performance on SNPs and indels is comparable. Extended Data Fig. 6 shows the F1 score for SNPs and indels across all GiaB stratifications with at least 100 variants in the truth set.

Extensibility of avidity sequencing

To assess the extensibility of avidity chemistry we continued a sequencing run beyond 150 bp to generate a 1 × 300 dataset from an *Escherichia coli* library. To achieve this we used both an optimized polymerase and an optimized reagent formulation. Figure 6a shows quality scores as a function of sequencing cycle. Because quality scores were not trained to these lengths, the scores are approximate. Figure 6b shows the *E. coli* error rate as a function of cycle number based on alignment to the known reference strain. The error rate of the final cycle was 1.9% and that at cycle 150 was 0.1%. Error calculations were based on the vast majority of the data with a pass filter rate for the run of >99.6% and Burrows–Wheeler aligner (BWA) settings aimed at strongly discouraging soft clipping (no cycles with soft clipping >0.04%). The enzymes and formulations developed for this run will be leveraged as we continue to identify extensions and improvements.

Discussion

We present a sequencing chemistry that achieves improved quality and lower reagent consumption by independent optimization of nucleotide incorporation and signal generation. Although other chemistries have proposed the separation of incorporation and signal generation[35], the avidite concept benefits from the fact that multiple nucleotides on the avidite bind multiple copies of the DNA template within a polony, which decreases dissociation rate constant and the labeled reagent concentration requirement for base classification. Furthermore, the avidite construct is modular. The core can be swapped for a different substrate. Both number and type of dye molecules are configurable, and many

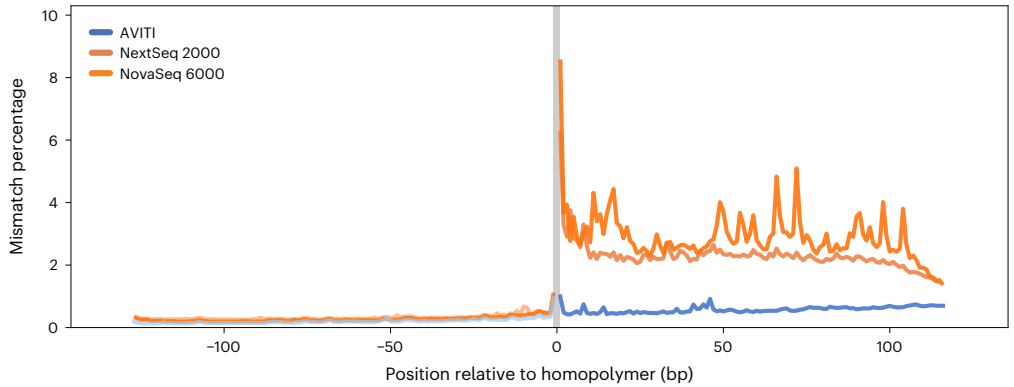

Fig. 4 | Post-homopolymer performance across platforms. Mismatch percentages of AVITI, NovaSeq 6000 and NextSeq 2000 reads before and after homopolymers of length 12 or greater.

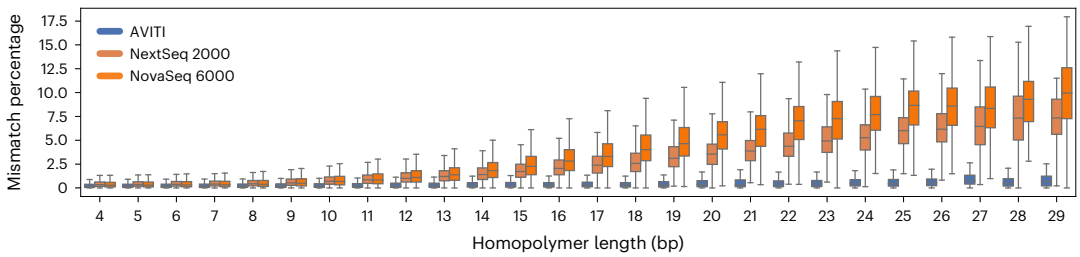

Fig. 5 | Comparison of mismatch rate following homopolymers of length between four and 29. Mismatch percentage difference between avidity sequencing and SBS increases with homopolymer length. The box plot shows median, quartiles and whiskers, which are 1.5× interquartile range.

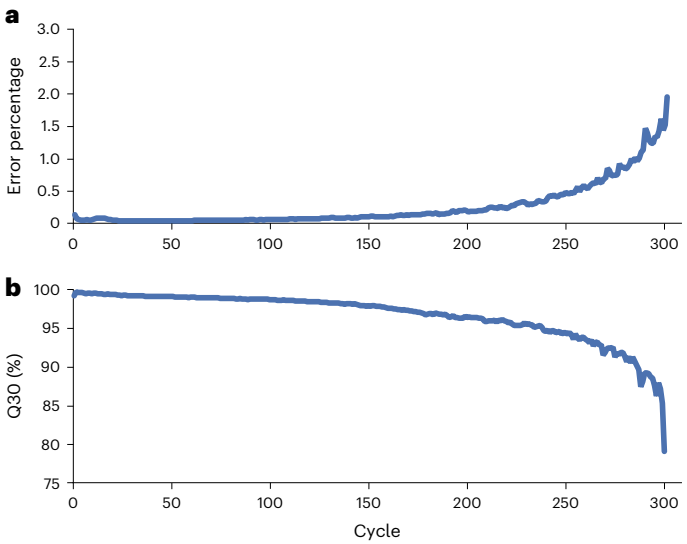

Fig. 6 | Performance of a 300-cycle *E. coli* sequencing run. a, Percentage *Q*30 by cycle. Overall *Q*30 percentage exceeds 96% and end of read has 85% *Q*30. **b**, *E. coli* error rate as a function of cycle. Alignment settings strongly discourage soft clipping, and >99% of reads pass filter. Final cycle error rate was 0.019.

types of linkers can be used. The changes are straightforward to implement and do not require modification of the polymerase responsible for binding the nucleotides attached to the linkers. The modular design speeds technology improvement because each component can be optimized in parallel for increased signal, decreased cycle time, lower reagent concentration or any other potential axis of improvement.

The avidity chemistry described above has been implemented as part of a benchtop sequencing solution. The accuracy of the sequencer was demonstrated by training a quality model on human sequencing data, which shows that in the majority of bases in an independent human-whole-genome sequencing run is >*Q*40. The high level of accuracy probably results from (1) the use of an engineered high-fidelity polymerase, (2) synergistic binding of multiple nucleotides on a single avidite to ensure only the correct cognate avidite binds to the polony and (3) a binding disadvantage for out-of-phase DNA copies within a polony that lack other out-of-phase neighbors to serve as avidity substrates. Future work will be required to investigate the relative contribution of each mechanism proposed above. In addition to overall accuracy improvements, the chemistry retains good performance in reads containing long homopolymers. The sequencer can be used in a wide range of applications, as exemplified by results for single-cell RNA-seq and for whole-human-genome sequencing. In both cases, reference standards were sequenced so that the quality of result could be assessed. The single-cell data exceeded the quality metric guidelines provided by 10X Genomics (https://www.10xgenomics.com/compatible-products?query=&page=1). The human genome variant-calling results showed high sensitivity and precision for both SNPs and small indels[36]. The two benchmarking studies were selected due to the availability of well-characterized samples and because they represent very different use cases. However, these are only examples and other applications have been demonstrated, including whole-genome sequencing for rare disease[37], low-pass sequencing with imputation[38] and single-cell sequencing of DNA and RNA[39]. Although the current implementation of avidity-based sequencing already achieves high accuracy and broad applicability, there are many improvement directions being explored. In addition to the initial demonstration of longer reads shown here, further quality improvements, shorter cycle times and higher densities are under development.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions

and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41587-023-01750-7.

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Methods

Solution measurements of nucleotide incorporation

Solution measurements of nucleotide kinetics were performed using commercially available dATP-Cy5 (Jena Bioscience, catalog no. NU-1611-CY5-S). DNA substrates for solution kinetic assays were prepared by annealing a 5′FAM-labeled primer oligo (purchased from IDT) and high-performance liquid chromatography-purified (5′-CGAGCCGTCCAACCTACTCA-3′) with a template oligo (5′-ACGACCATGTTGAGTAGGTTGGACGGCTCG-3′). Annealing was performed with 10% excess template oligo in the annealing buffer using a PCR machine to heat oligos to 95 °C, followed by slow cooling to room temperature over 60 min. Solution kinetics were performed by mixing a preformed enzyme–DNA complex with fluorescent nucleotide and $MgSO_4$ using a RQF3 Rapid Quench Flow (KinTek Corp.). The enzyme used was an engineered variant of *Candidatus altiarchaeales* archaeon. The final reaction was conducted in 25 mM Tris pH 8.5, 40 mM NaCl and 10 mM ammonium chloride at 37 °C. Extension products were separated from unextended primer oligos by capillary electrophoresis using a 3500 Series Genetic Analyzer (ThermoFisher) to achieve single-base resolution. Products were quantified and fit to a single exponential equation. The observed rates as a function of nucleotide concentration were then fit to a hyperbolic equation to derive apparent K_d ($K_{d,app}$) and rate of polymerization (k_{pol}).

Avidite synthesis and construction

Initial research scale avidites were constructed by dissolving 5 mg of 10 kD 4-arm-PEG-SG (Laysan Bio, catalog no. 4arm-PEG-SG-10K-5g) in 100 µl of 95% organic solvent (for example, ethanol) and 5 mM MOPS pH 8.0 to make a 50 mg ml^{-1} solution (5 mM), 19 µl of which was combined with 1.5 µl of 10 mM dATP-NH_2 (7-deaza-7-propargylamin′-2′-deoxyadenosin′-5′-triphosphate; Trilink, catalog no. N-2068) and 8.0 µl of 3.75 mM 2 kD Biotin-PEG-NH_2 (Laysan Bio, catalog no. Biotin-PEG-NH2-2K-1g) in 95% organic solvent (for example, ethanol) and 5 mM MOPS pH 8.0. After mixing, 5 mM 10 kD 4-arm-PEG-SG was added. The final composition was 0.50 mM dA-NH_2, 1.0 mM biotin-PEG-NH2 (2 kD), 0.25 mM 4-arm-PEG-NHS, 85.5% organic solvent (for example, ethanol) and 4.5 mM MOPS pH 8.0. Following 1,000-rpm incubation at 25 °C for 90 min, the reaction volume was adjusted to 100 µl by the addition of MOPS pH 8.0. Purification was performed using a Biorad Biospin P6 column pre-equilibrated in 10 mM MOPS pH 8.0. The purified dATP-PEG–biotin complex was mixed with Zymax Cy5 Streptavidin (Fisher Scientific, catalog no. 438316) in a 2.5:1 volumetric ratio and allowed to equilibrate for 30 min at room temperature.

Real-time measurement of avidite association and dissociation

Real-time measurement of avidite binding kinetics was performed using an Olympus IX83 microscope at 545 and 635 nm excitation (Lumencor Light Engine) set to an approximate power density of about 1 W cm^{-2}, with an Olympus objective (catalog no. UCPLFLN20XPH) and a Semrock BrightLine multiband laser filter set (catalog no. LF405/488/532/635) containing a matching quad band exciter, emitter and dichroic. Flow rates of 60 µl s^{-1} were used for reagent exchanges. Circular PhiX libraries were introduced to AVITI flow cells, hybridized in 3× SSC buffer for 5 min at 50 °C and cooled to room temperature. Amplification reagents were introduced into the flow cell to perform rolling circle amplification and amplify genomic DNA. The instrument was paused following polony generation and priming and the flowcell moved to the microscope. Custom control software was written to control all peripheral hardware and synchronize data collection with flow of materials into the sample. Data collection (4 fps) was triggered by flow of the avidity mix and collected for 55 s. Polonies in the field were localized by a spot-finding algorithm, and background-corrected intensities were extracted versus time. Experiments were performed at 0.5 pM, 1 nM, 7.5 nM and 10 nM avidite or monovalent dye-labeled nucleotide concentrations. Substrates at the respective concentrations were combined with 100 nM engineered enzyme variant of *C. altiarchaeales* archaeon in the avidity on rate assay buffer formulation (25 mM HEPES pH 8.8, 25 mM NaCl, 0.5 mM EDTA, 5 mM strontium acetate, 25 mM ascorbic acid and 0.2% Tween-20). Avidites and nucleotides were labeled with Alexa Fluor 647. Higher-concentration data collection was limited by the ability to detect polony intensity from free avidite intensity at elevated concentrations. Off-rate measurements were performed by binding avidites to flowcell polonies, followed by washing with avidity on rate assay buffer and triggering of data collection.

Genomic DNA and next-generation sequencing library preparation

Human DNA from cell line sample HG002 was obtained from the Coriell Institute. Linear next-generation sequencing library construction was performed using a KAPA HyperPrep library kit (Roche, catalog no. 07962363001) according to published protocols. Finished linear libraries were circularized using the Element Adept Compatibility kit (catalog no. 830-00003). Final circular libraries were quantified by quantitative PCR with the standard and primer set provided in the kit. Circular library DNA was denatured using sodium hydroxide and neutralized with excess Tris pH 7.0 before dilution. Denatured libraries were diluted to 8 pM in hybridization buffer before loading onto the sequencing cartridge.

Single-cell 3′ gene expression library circularization

Single-cell RNA-seq libraries were prepared from two lots of peripheral blood mononuclear cell suspension (10,000 and 1,000 cells) using the Chromium Next GEM Single Cell 3′ Kit v.3.1 (catalog no. 1000268). Each library was quantified and individually processed for sequencing using the Adept Library Compatibility Kit (catalog no. 830-00003). Processed libraries were pooled and sequenced with 28 cycles for read 1, 90 for read 2 and index reads.

Sequencing instrument and workflow

Sequencing results were obtained with commercialized formulations of avidites, enzymes and buffers. Element Bioscience's AVITI commercial system (catalog no. 88-00001) was used for all sequencing data. AVITI 2 × 150 kits were loaded on the instrument (catalog no. 86-00001). Primary analysis was performed onboard the AVITI sequencing instrument, and FASTQ files were subsequently analyzed using a secondary analysis pipeline from Sentieon.

Sequencing primary analysis

Four images were generated per field of view during each sequencing cycle, corresponding to the dyes used to label each avidite. An analysis pipeline was developed that uses the images as input to identify the polonies present on the flowcell and to assign to each polony a base call and quality score for each cycle, representing the accuracy of the underlying call. The analysis approach has steps similar to those described in ref. 25. Briefly, intensity is extracted for each polony in each color channel; intensities are then corrected for color cross-talk and phasing and normalized to make cross-channel comparisons. The highest normalized intensity value for each polony in each cycle determines the base call. In addition to assigning a base call, a quality score corresponding to call confidences is also assigned. The standard Q-score definition is utilized where the Q-value is defined as $Q = -10 \times \log\_10p$, where p is the probability that the base call is an error. Q-score generation follows the approach of Ewing et al., with modified predictors[21], and is encoded using the phred+33 ASCII scheme. The predictors used for quality score training are (1) maximum intensity per polony across color channels; (2) clarity of each polony (defined as $(A + 1)/(B + 1)$, where A is the highest intensity across color channels and B is the second highest); (3) the sum

of phasing and prephasing estimates; and (4) the median clarity value taken across the 10% of the lowest-intensity polonies. The sequence of base call assignments and quality scores across the cycles constitutes the output of the run. These data are represented in standard FASTQ format for compatibility with downstream tools.

Quality score assessment

To assess the accuracy of quality scores (Fig. 3), the FASTQ files were aligned with BWA to generate BAM files. GATK BaseRecalibrartor was then applied to the BAM, specifying files of publicly available known sites to exclude human variant positions.

K-mer error analysis

The same run used to generate recalibrated quality scores was analyzed via custom script for all *k*-mers of size 1, 2 and 3. The computation is based on 1% of a 35X genome to ensure adequate sampling of each *k*-mer. For example, each 3-mer is sampled at least 850,000 times (average 6.7 million). This figure is based on a publicly available run from each platform. For the instances of each *k*-mer, the percentage mismatching a variant-masked reference was computed. The same script was applied to a publicly available NovaSeq dataset for HG002 and a publicly available NextSeq 2000 dataset for HG001 (Demo Data for HG002 were not available). We tabulated the number of *k*-mers in which the percentage incorrect was lowest for AVITI among the three platforms compared.

Homopolymer analysis

A BED file provided by National Institute of Standards and Technology (NIST) genome-stratifications v.3.0, containing 673,650 homopolymers of length >11, was used to define regions of interest for homopolymer analysis (GRCh38_SimpleRepeat_homopolymer_gt11_slop5). Reads overlapping these BED intervals (using samtools view -L and adjusting for slop5) were selected for accuracy analysis. Reads with any of the following flags set were discarded: secondary, supplementary, unmapped or reads with mapping quality of 0. Reads were oriented in the 5′→3′ direction and split into three segments: preceding the homopolymer, overlapping it and following it. The mismatch rate for each read segment was computed, excluding N-calls, softclipped bases and indels. For example, if a 150-bp read (aligned on the forward strand) contained a homopolymer in positions 100–120, the first 99 cycles were used to compute the error rate before the homopolymer and the last 30 to compute error rate following the homopolymer. Reads were discarded if the sequence either preceding or following the homopolymer was <5 bp in length. All reads were then stacked into a matrix according to their positional offset relative to the homopolymer, and error rate per post-offset was computed.

Average error rate was computed for avidity sequencing runs and for publicly available data from multiple SBS instruments, for comparison. Differences oin mismatch percentage, across all BED intervals, between AVITI and NovaSeq were plotted in a histogram and examples showing various percentiles within the distribution were chosen for display via Integrative Genomics Viewer.

Publicly available datasets for NovaSeq were obtained from the Google Brain Public Data repository on Google Cloud (https://console.cloud.google.com/storage/browser/brain-genomics-public/research/sequencing/fastq). Publicly available NextSeq 2000 data were obtained from Illumina Demo Data on BaseSpace (https://basespace.illumina.com/datacentral).

Single-cell gene expression data analysis

Following sequencing, Bases2Fastq software was used to generate FASTQ files for compatible upload into 10X Cloud and subsequent analysis with the 10X Genomics Cell Ranger analysis package. Data visualization of single-cell gene expression profiling was generated using 10X Genomics Loupe Browser.

Whole-genome sequencing analysis

A FASTQ file with base calls and quality scores was downsampled to 35× raw coverage (360,320,126 input reads) and used as an input into Sentieon BWA followed by Sentieon DNAscope[40]. Following alignment and variant calling, variant calls were compared with the NIST genome in Bottle Truth Set v.4.2.1 via the hap.py comparison framework to derive total error counts and F1 scores[41]. The results are computed based on the 3,848,590 SNV and 982,234 indel passing variant calls made by DNAScope.

1 × 300 Data generation

An *E. coli* library was prepared using enzymatic shearing and PCR amplification. The library was then sequenced for 300 cycles using new enzymes for stepping along the DNA template and for avidite binding. The reagent formulation with increased enzyme and nucleotide concentrations during the stepping process was used to improve stepping performance. The contact times for avidite binding and exposure were both reduced without performance losses, to decrease cycle time over the 600 cycles of sequencing. The displays show only 299 cycles of data, because cycle 300 was used only for prephasing correction. To minimize soft clipping during alignment the following inputs were used in the call to BWA–MEM: -E 6,6 -L 1000000 -S.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The avidity sequencing datasets described in the paper are available for download via the AWS CLI in the public bucket s3://avidity-manuscript-data/, pending upload to the sequence read archive under BioProject PRJNA869673. Publicly available datasets for NovaSeq were obtained from the Google Brain Public Data repository on Google Cloud (https://console.cloud.google.com/storage/browser/brain-genomics-public/research/sequencing/fastq). Publicly available NextSeq 2000 data were obtained from Illumina Demo Data on BaseSpace (https://basespace.illumina.com/datacentral).

Code availability

Scripts used for analysis are available via GitHub (https://github.com/Elembio/AvidityManuscript2023).

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Author contributions

The author list is divided into three sections, each in alphabetical order. Authors in the first section made equal contributions to the critical elements of the technology and paper development. Authors in the second category made specific technology contributions described within the paper. Authors in the third group helped to develop some aspects of the underlying technology that culminated in the final product. M.H. and M.P. shared in the intellectual supervision of the work.

Competing interests

All authors are current or former employees of Element Biosciences. All authors may hold stock options in the company.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41587-023-01750-7.

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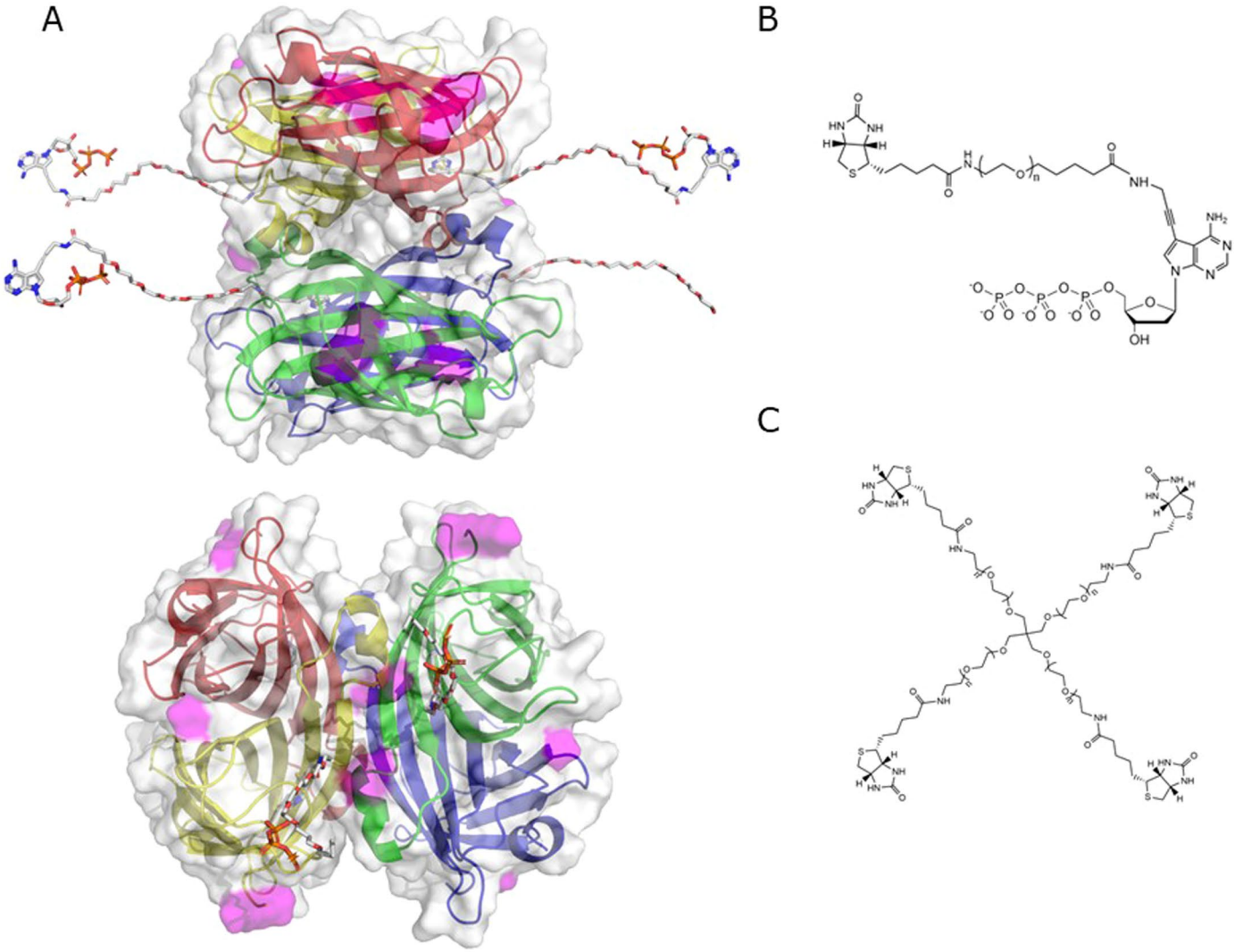
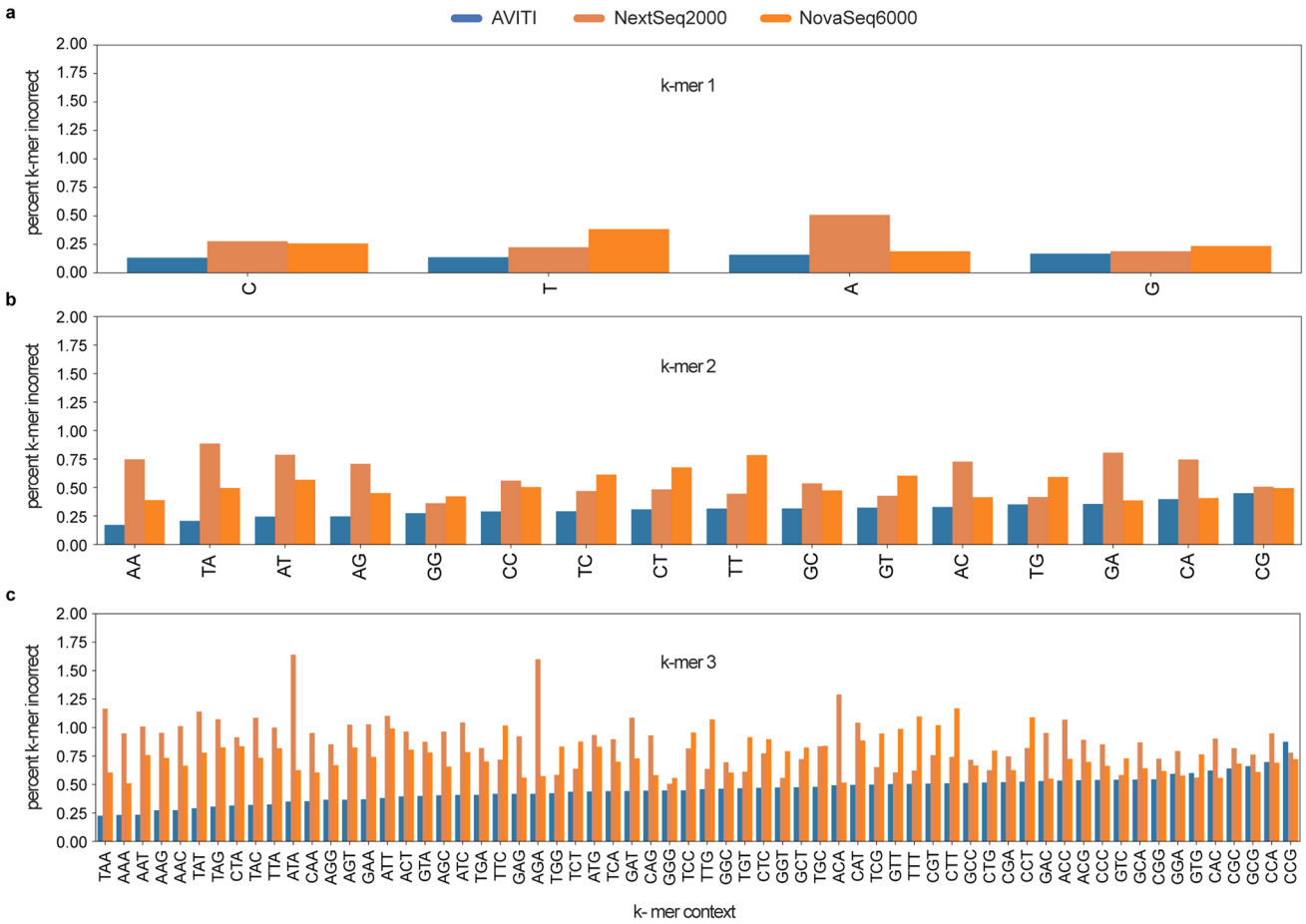
https://doi.org/10.1038/s41587-023-01750-7



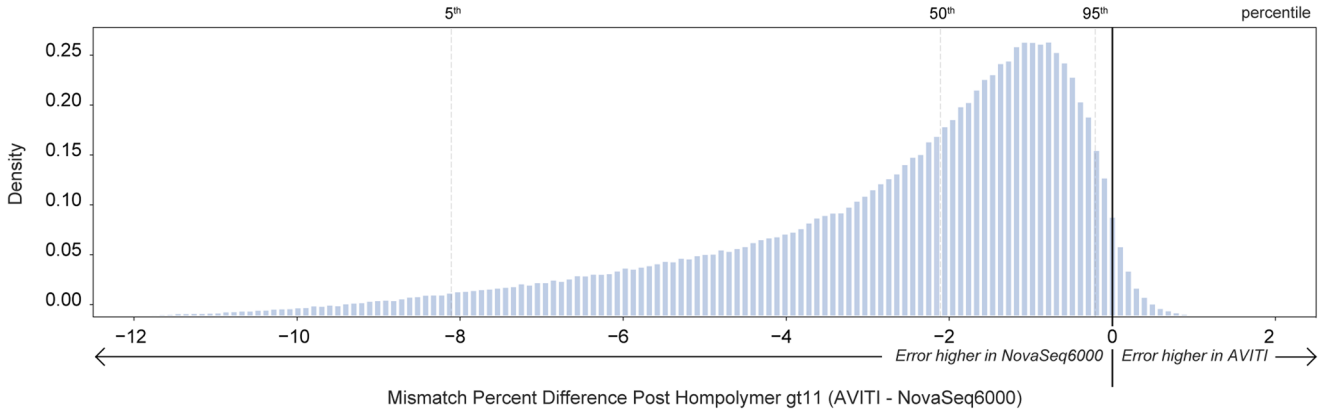
Extended Data Fig. 1 | Model of an avidite. (**a**) side and top views of a modeled avidite. The protein core consists of fluorophore labeled streptavidin. The monomers of tetrameric streptavidin are colored red, blue, green, and yellow. Dye conjugation sites through lysine-NHS chemistry are denoted in the surface rendering as magenta. Fluorophores are not pictured. Avidite arms are associated via a biotin interaction with the core streptavidin protein. Arms are mixed stoichiometrically to achieve averages of three nucleotide containing arms and one linker to additional cores. Molecules conjugated to have been shortened in this representation. (**b**) Structure of an avidite arm. (**c**) Structure of the 4-arm linker connecting avidite cores.

https://doi.org/10.1038/s41587-023-01750-7



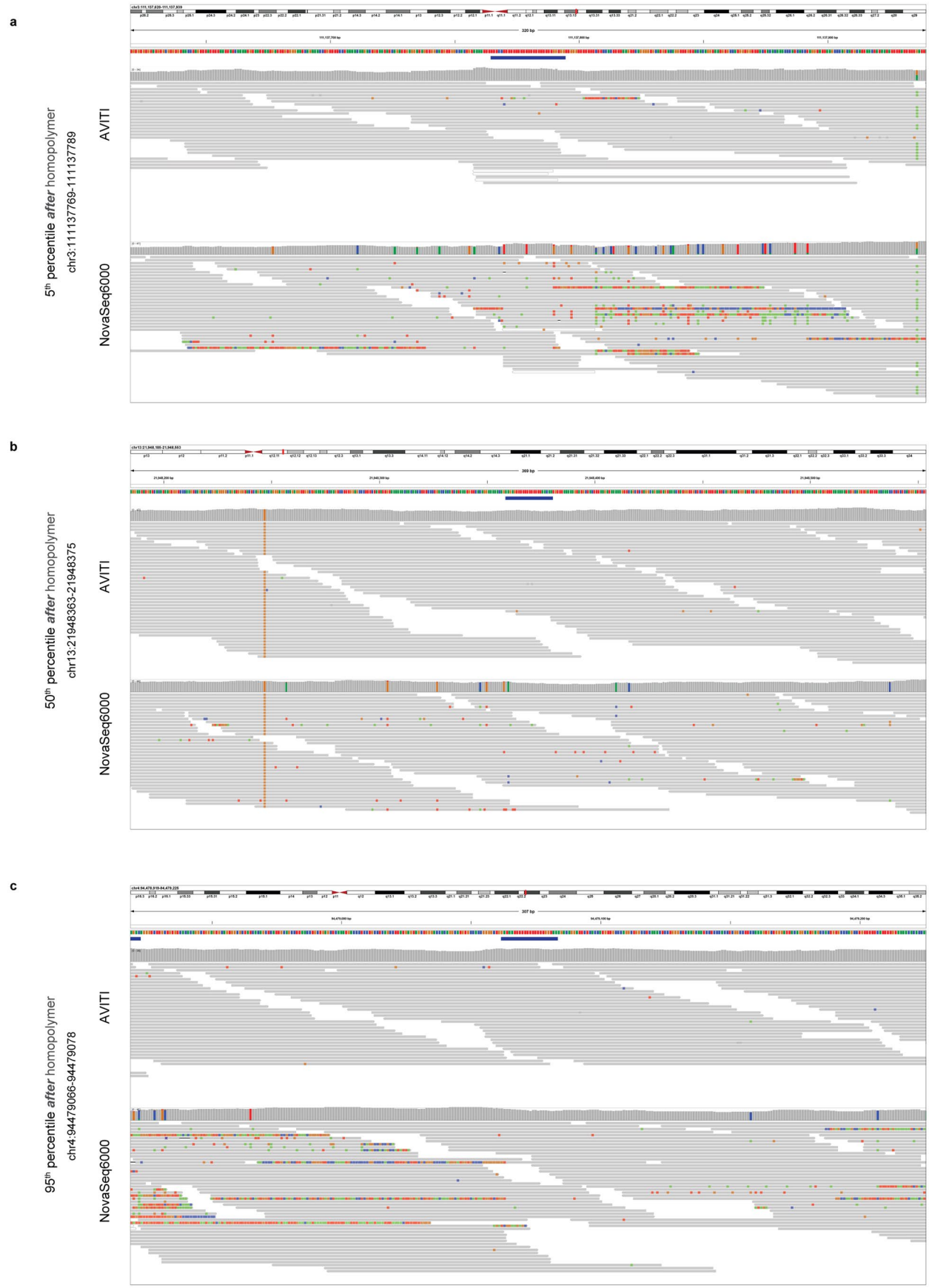
Extended Data Fig. 2 | Percentage of instances that a k-mer contained at least one mismatch compared across 3 instruments. Panels **a**, **b**, and **c** display 1-mers, 2-mers, and 3-mers, respectively. The bars are sorted by AVITI contexts from most to least accurate.

https://doi.org/10.1038/s41587-023-01750-7



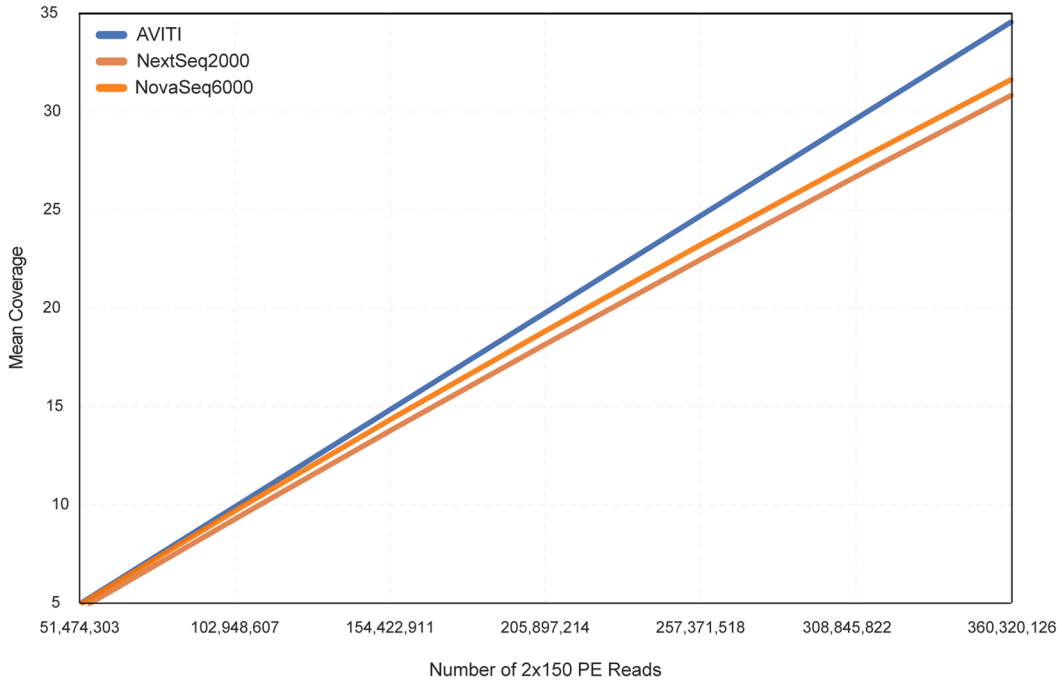
Extended Data Fig. 3 | Histogram of pairwise error differences. Difference was selected as the metric to cancel the effects of human variants from the mismatch percent.

https://doi.org/10.1038/s41587-023-01750-7



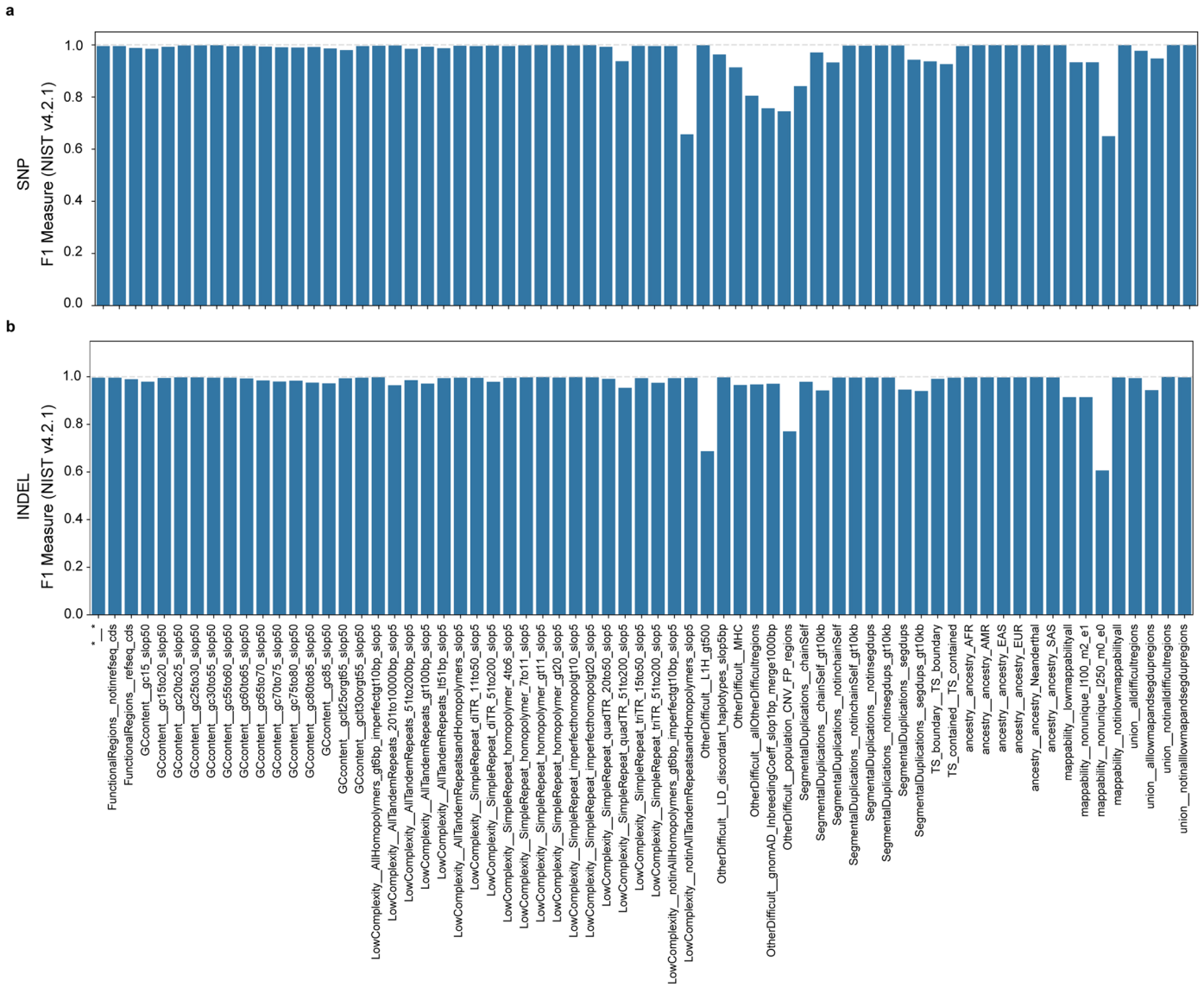
Extended Data Fig. 4 | IGV display of homopolymer loci at the 5th, 50th, and 95th percentile of AVITI minus NovaSeq mismatch percent (corresponding to the dashed lines of Extended Data Fig. 3). The red bar at the top indicates the homopolymer. Colors within the IGV read stack correspond to mismatches and softclipping. Only mismatches contribute to the error rate calculation and softclipped bases are ignored.

https://doi.org/10.1038/s41587-023-01750-7



Extended Data Fig. 5 | Comparison of read number vs genomic coverage computed via Picard for PCR-free whole genome data. AVITI most closely matches the 45-degree line due to the low duplicate rate.

https://doi.org/10.1038/s41587-023-01750-7



Extended Data Fig. 6 | F1 Score of SNPs and indels across GiaB stratifications. F1 score for SNPs and indels stratified by all GiaB regions with at least 100 variants in the 4.2.1 truth set of sample HG002.

https://doi.org/10.1038/s41587-023-01750-7

Extended Data Table 1 | Single cell expression: CellRanger metric values for 10 K cell and 1 K cell libraries from the PBMC reference

CellRanger v7.0 Metric	Performance expectation	AVITI 10K cells	AVITI 1K cells
Valid barcodes	>90%	97.5%	97.5%
Reads mapped confidently to exonic regions	>50%	53.0%	53.8%
Read mapped confidently to transcriptome	>40%	74.7%	77.8%
Fraction reads in cells	>80%	95.5%	92.6%
Q30 bases in barcode	>85%	99.5%	99.5%
Q30 bases in RNA read	>75%	98.6%	98.8%
Mean reads per cell	>50,000	61,326	68,766
Median genes per cell	>1700	2,910	2,951
Total genes detected	N/A	23,863	29,679
Estimated number of cells	+/-20%	8,513	922

https://doi.org/10.1038/s41587-023-01750-7

Extended Data Table 2 | Variant calling performance for HG002 on GIAB-HC regions

	Sensitivity	Precision	F1-Score
SNP	0.9939	0.9977	0.9958
Small indel	0.9928	0.9980	0.9954

nature portfolio

Corresponding author(s): Michael Previte

Last updated by author(s): 3/7/2023

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Kinetic data for Figure 2A was collected using RQF3 Rapid Quench flow (Kintek corporation). Real-time measurements for Figure 2B-E were collected on an Olympus IX83 microscope equipped with 545 and 637 lines (Lumencor), Semrock brightline multiband laser filter set (LF405/488/532/635) containing matching quad band exciter, emitter and dichroic. Flow was induced by a syringe pump pulling reagents across an AVITI flow cell at a rate of 60 ul/s. Prior to injection of reagents, real-time data was collected on an Andor sCMOS camera at 4 frames/s. All sequencing data was collected on the AVITI commercial instrument.
Data analysis	Kinetic data was analyzed and fit using conventional non-linear regression. All error bounds were propagated in the analysis and are reflected in figure 2 panel A. Reported kcat and Kd,app were obtained by fitting to a hyperbolic equation using no constrains other than the error reported for each point. Primary analysis of the collected data was performed on the AVITI instrument according to similar steps described on Whiteford et al. (25) FASTQ were generated using the bases2fastq software toolkit (version 1.1.1). Tools and scripts supporting bioinformatic analysis of this manuscript can be found at the following repo located on github - https://github.com/Elembio/AvidityManuscript2023. Single cell RNA was performed using CellRanger (version 7.0.1). Whole genome sequencing analysis was performed by first down-sampling the input FASTQ to 35X raw coverage (360,320,126, 2x150 input reads), and then aligning, de-duplicating and sorting using sentieon bwa (version 202112.02). The BAM was then used as input to Sentieon DNAscope (version 202112.02) in addition to a element specific ML model (SentieonDNAscopeModelElementBio0.3.model) to produce a VCF. Following alignment and variant calling, the variant calls were benchmarked using hap.py (version hap.py-0.3.14) to the NIST genome in a bottle truth set v4.2.1 across all regions to derive total error counts and F1 scores. To assess the accuracy of quality scores shown in Fig. 3, the aligned BAMS were processed using GATK BaseRecalibrartor (version gatk4:4.2.0.0—0), and specifying publicly available known sites files to exclude human variant positions (HG002 NIST v4.2.1 bed/vcf,

10206_phase1.snps.high_confidence.hg38, dbsnp_144.hg38). The resulting predicted and recalibrated q-scores were plotted.
To compute the mismatch percentage of AVITI, NovaSeq 6000 and NextSeq 2000 reads before and after homopolymers of length 12 or greater, a BED file provided by NIST genome-stratifications v3.0, containing 673,650 homopolymers of length greater than 11 was used to define the regions of interest for the homopolymer analysis (GRCh38_SimpleRepeat_homopolymer_gt11_slop5). Reads that overlapped these BED intervals (using samtools version 1.1.1) were selected for accuracy analysis. Reads with any of the following flags set were discarded (secondary, supplementary, unmapped or reads with mapping quality of 0). Reads were oriented in the 5' -> 3' direction, and split into 3 segments, preceding the homopolymer, overlapping the homopolymer, and following the homopolymer. The mismatch rate for each read-segment was computed, excluding N-calls, softclipped bases and indels. For example, if a 150 bp read (aligned on the forward strand) contains a homopolymer in positions 100-120, then the first 99 cycles were used to compute the error rate prior to the homopolymer, and the last 30 cycles were used to compute the error rate following the homopolymer. Reads were discarded if either the sequence preceding or following the homopolymer was less than 5bp in length (accounting for the GIAB slop used). All reads were then stacked into a matrix, according to their positional offset relative to the homopolymer, and error rate per pos-offset was computed.
The average error rate was computed for avidity sequencing runs and for publicly available data from multiple SBS instruments, for comparison. The differences of mismatch percentages, across all BED intervals, between AVITI™ and NovaSeq were plotted in a histogram and examples showing various percentiles within the distribution were chosen for display via IGV.

The interval-error.tsv and offset-error.tsv files can be found in the following directory: https://github.com/Elembio/AvidityManuscript2023/tree/main/data/homopolymer-error/GRCh38_SimpleRepeat_homopolymer_gt11_slop5
To compute the mismatch percent difference between avidity sequencing and SBS across homopolymer lengths, the four GIAB supplied homopolymer bed files were combined, and duplicates were removed (4to6, 7to11, gt11, gt20), producing a new bed file representing all homopolymer of size 4 to inf. The box plot shows median, quartiles, and the whiskers are 1.5*IQR.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The avidity sequencing data sets described in the manuscript are available for download via the AWS CLI using the following command:
aws s3 ls --no-sign-request s3://avidity-manuscript-data/

Samples and FASTQ have been accessioned in SRA under BioProject PRJNA869673.

Bioinformatic tools and scripts can be found on the following github repo: https://github.com/Elembio/AvidityManuscript2023

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	There were no human research participants in this study.
Population characteristics	There were no human research participants in this study.
Recruitment	There were no human research participants in this study.
Ethics oversight	There were no human research participants in this study.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sequencing calibration studies were performed on 20 samples. Single cell studies were performed on multiple samples that generated consistent results, but a single example was used for this particular study. To determine k-mer errors, a million k-mers of each length were used to determine percent mismatch. For the homopolymer analysis, ~700,000 loci were used. For GiaB stratifications, we selected context classes with at least 100 variants.
Data exclusions	There was no data excluded (Filtered data is excluded from the sequencing runs).
Replication	We checked that all presented runs are representative by looking at no fewer than 20 sequencing runs. For analyses such as homopolymer and k-mer accuracy, sample size calculations are based on the number of relevant loci within a run. There were no failures to replicate.
Randomization	The study performed was validating first principles studies such as enzyme kinetics to validate the hypotheses of avidity chemistry, thus sample randomization would not be necessary. Sequencing data was performed on known samples and comparative metrics to known reference samples also obviates the need for randomization of the studies as the known reference samples are a widely known control.
Blinding	The study performed was validating first principles studies such as enzyme kinetics to validate the hypotheses of avidity chemistry, thus blind would not be necessary. Sequencing data was performed on known samples and comparative metrics to known reference samples also obviates the need for blind studies as the known reference samples are a widely known control.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

EXHIBIT 22

Element Biosciences

Element AVITI™ System

FOR USE WITH

Element AVITI System, catalog # 880-00001
AVITI 2x75 Sequencing Kit Cloudbreak™, catalog # 860-00004
AVITI 2x150 Sequencing Kit Cloudbreak, catalog # 860-00003
AVITI 2x75 Sequencing Kit, catalog # 860-00002
AVITI 2x150 Sequencing Kit, catalog # 860-00001
AVITI Operating Software v2.0.0 or later

Document # MA-00008 Rev. D
April 2023

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Table of Contents

CHAPTER 1

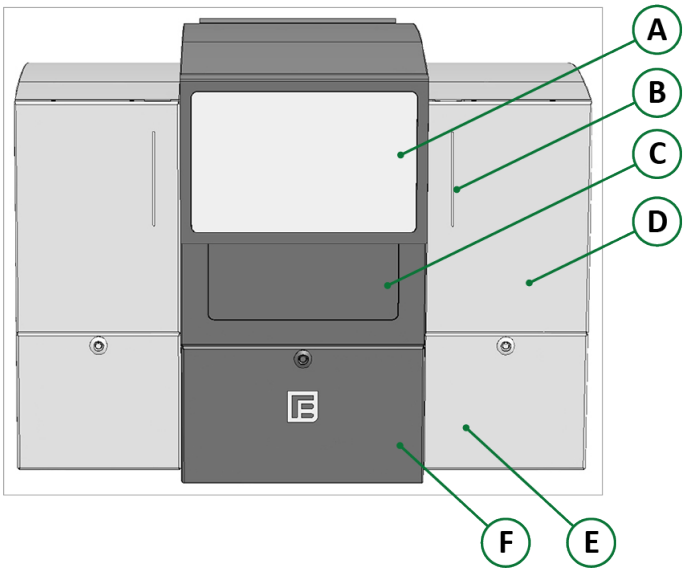
System Overview

Introduction

The Element AVITI System is a mid-throughput next-generation sequencing (NGS) system that amplifies and sequences DNA libraries. The system is divided into side A on the left and side B on the right. Each side operates independently so you can engage one side while the other is in use. A touchscreen monitor displays the AVITI Operating Software (AVITI OS).

This workflow guide provides a system overview and comprehensive instructions for safely operating and maintaining the instrument.

Figure 1: AVITI System overview



A Touchscreen monitor
B Lightbars for each side show system status
C Nest bay contains the flow cells
D Pump bays hold the pumps
E Reagent bays hold reagents and wash solution
F Waste bay holds waste bottles

Site Prep and Safety

The instrument does not contain any user-servicable parts. Software and interlocks prevent exposure to hazards, but using the AVITI System in an unspecified manner can compromise these protections. If performance is not to published specifications, contact Element Technical Support.

Before operating or maintaining the instrument, meet the site requirements detailed in the *Element AVITI System Site Prep Guide (MA-00007)*. Review the safety and regulatory information detailed in *Safety and Compliance* on page 90.

System Compatibility

The AVITI System is compatible with single-strand DNA (ssDNA) libraries prepared with the Element Adept™ Library Compatibility Workflow, Element Elevate™ Library Prep Workflow, 16S LoopSeq™ for AVITI, or Amplicon LoopSeq for AVITI. LoopSeq for AVITI libraries include Elevate indexes and adapters and are therefore considered Elevate libraries.

The AVITI OS supports the chemistry combinations presented in the following table. The AVITI 2x75 Sequencing Kit and AVITI 2x150 Sequencing Kit use version 1 chemistry. Cloudbreak kits use Cloudbreak chemistry. To avoid mixing and matching components from different kit configurations and versions, AVITI OS validates the compatibility of the cartridge and flow cell provided in each sequencing kit.

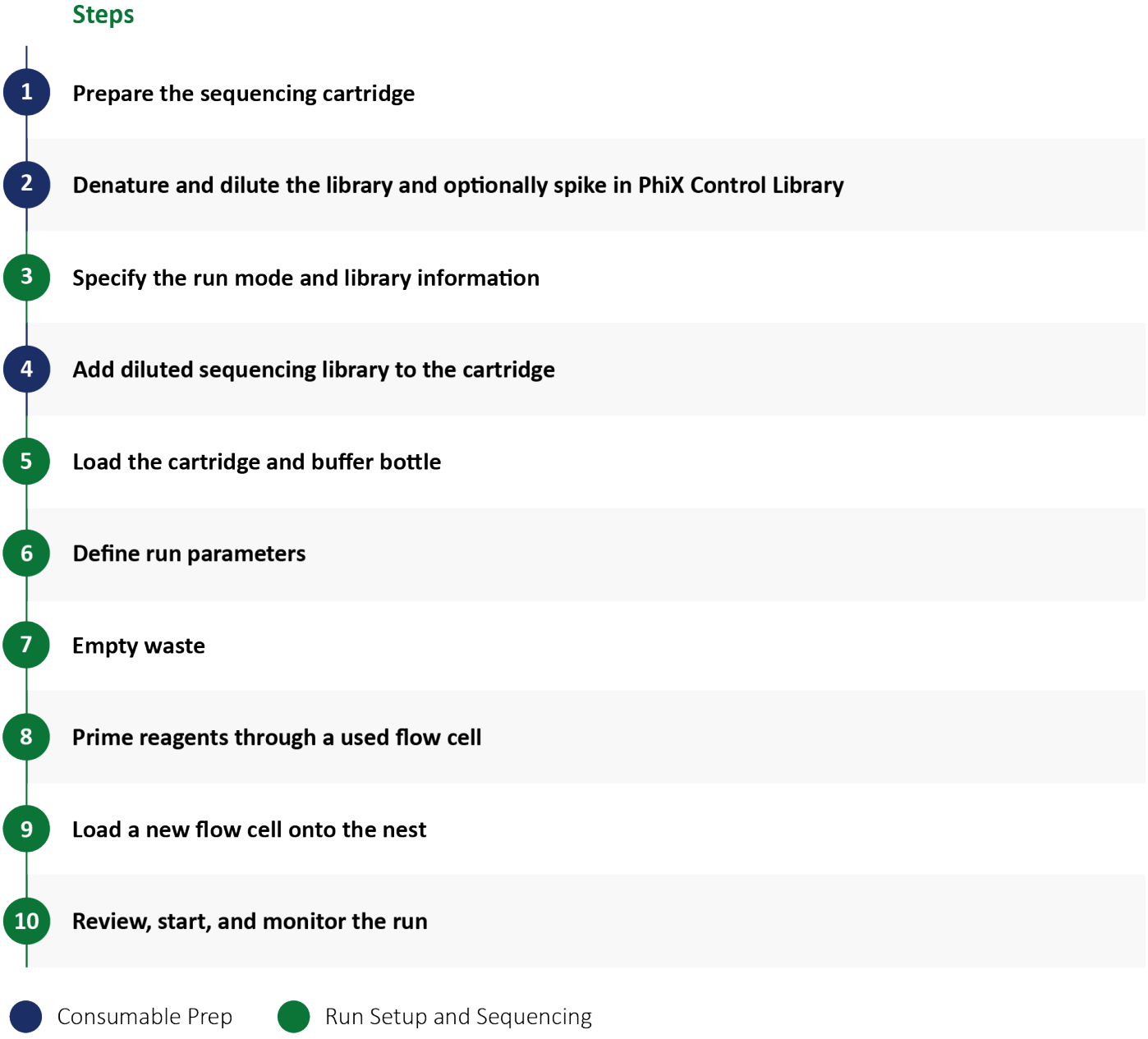
Table 1: Supported chemistry versions

Library Prep	Sequencing Kits*	Control Library	Custom Primers
Adept Workflow (v1.1 kits)	• AVITI 2x75 Sequencing Kit • AVITI 2x150 Sequencing Kit	PhiX Control Library, Adept	Adept Custom Oligonucleotide Buffer Set
	• AVITI 2x75 Sequencing Kit Cloudbreak • AVITI 2x150 Sequencing Kit Cloudbreak	PhiX Control Library, Adept	Adept Custom Primer Set Cloudbreak
Elevate Workflow	• AVITI 2x75 Sequencing Kit • AVITI 2x150 Sequencing Kit	PhiX Control Library, Elevate	Not applicable
	• AVITI 2x75 Sequencing Kit Cloudbreak • AVITI 2x150 Sequencing Kit Cloudbreak	Cloudbreak PhiX Control Library, Elevate	Not applicable

Workflow Summary

The following figure summarizes the steps to sequence on the AVITI System, including preparing diluted sequencing library and other consumables and setting up the run. Diluted sequencing library is a library or library pool at the appropriate volume and concentration for sequencing.

Figure 2: Overview of the sequencing workflow



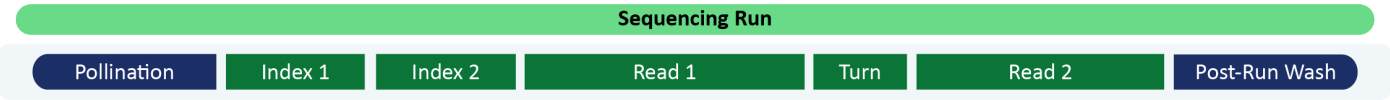
Run Stages

AVITI OS generates a recipe based on run parameters entered during run setup. The recipe governs each stage of the run. A run is complete when the recipe is executed and primary analysis is done.

The following stages comprise a run:

- **Amplification**—Hybridizes the library to the flow cell and forms polonies, each containing multiple copies of the same sequence from the library.
- **Sequencing**—Performs each read in the run, including imaging and primary analysis.
- **Post-run wash**—Automatically flushes buffer from the sequencing cartridge through the fluidic system to remove salts and residual library.

Figure 3: Cloudbreak recipe for a paired-end run with indexing



Reads in a Run

Up to four reads comprise a run:

- **Index 1** sequences the Index 1 sequence and **Index 2** sequences the Index 2 sequence. A dual-index run performs both index reads, a single-index run performs Index 1 only, and a nonindexed run skips both index reads.
- **Read 1** sequences the forward strand of the DNA insert and is always required. Starting from the opposite end of the insert, **Read 2** sequences the reverse strand. A paired-end run performs both reads, including a paired-end turn before Read 2 to generate the complementary strand. A single-read run performs Read 1 only.

Number of Cycles

Read length is the total number of cycles a run includes. A 2 x 75 or 2 x 150 kit configuration indicates the number of cycles that sequence the DNA insert. You can distribute the total cycles depending on experimental design.

The optimal number of cycles to perform in a run depends on your experiment, but the software and chemistry prescribe a minimum and maximum. Read 1 requires at least five cycles to perform a run and at least 25 cycles to generate all run metrics. Elevate Cloudbreak libraries require at least four Index 1 cycles. The maximum number of cycles depends on configuration:

- A 2 x 75 kit sequences up to 184 cycles, supporting one 2 x 76 run with indexing and unique molecular identifiers (UMIs).
- A 2 x 150 kit sequences up to 334 cycles, supporting one 2 x 151 run with indexing and UMIs.

CHAPTER 2

Instrument Hardware

AVITI System Hardware

The instrument is divided into side A on the left and side B on the right. Each side operates independently so you can engage one side while the other is in use. The middle of the instrument includes a glove-compatible touchscreen that displays the AVITI OS interface. Internally, a camera and four tube lenses image the flow cell in four channels.

Status Lights

The AVITI System includes two types of status lights: an exterior lightbar on each side of the instrument and an interior nest light in front of each nest. The lightbar colors indicate the current process and overall system status and the nest light colors indicate flow cell status.

Unless the system is initializing, each lightbar is side-specific. The color immediately changes when an error occurs. For a warning, the color changes after the run finishes.

Lightbar Colors

Color	Status
White fade	The system is initializing.
Solid white	The system is initialized and idle.
Solid blue	Run or wash setup is in progress.
Blue fade	The system is priming, sequencing, or washing.
Solid orange	The system experienced a warning.
Solid red	The system experienced an error or run failure.

Nest Light Colors

Color	Status
Blue	The flow cell is present and ready to be unloaded.
Green	The flow cell is properly loaded and ready for priming, sequencing, or washing.
Red	The flow cell is improperly loaded: the lid is open or the nest is empty.
None	The flow cell is present but is not ready to be unloaded.

Exterior Shells

Exterior shells enclose the instrument to maintain internal temperatures, exclude dust and other external elements, and protect operators from exposure to lasers, mechanical moving parts, and other internal hazards. For more information, see *Safety and Compliance* on page 90.

Instrument Bays

Instrument bays hold consumables and accessories for runs and washes. Each side includes a dedicated reagent bay and a dedicated pump bay. Bays in the middle of the instrument hold flow cells and waste for both sides.

Lighting illuminates the interior of each bay. During a run, AVITI OS locks all doors except the pump bay doors to protect against lasers, mechanical moving parts, and other hazards.

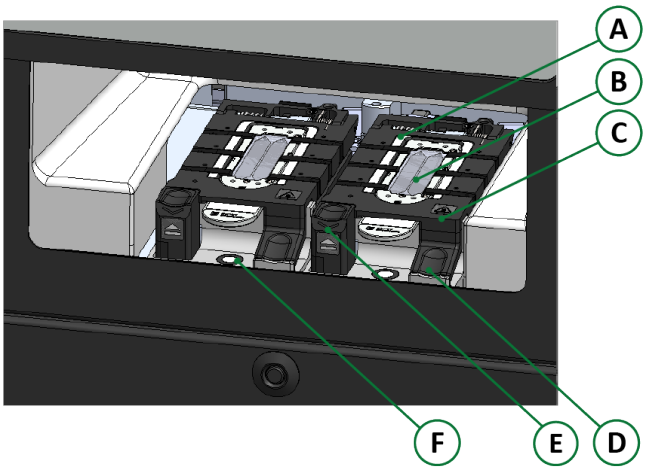
CAUTION
Do not place items on top of the instrument or open doors. The doors can support the weight of run and wash components but leaning on or bumping into a door can damage the instrument.

Nest Bay

The nest bay includes two nests, one for each side. Each nest holds one flow cell secured with a lid. A button unlatches and opens the lid to a 40° angle. A tab closes the lid and secures the flow cell.

To guide placement of the flow cell onto the nest, a wall encircles the loading area. Three silver pins fit into three corresponding holes on the flow cell cartridge, ensuring proper alignment and seating. An automated door encloses the bay.

Figure 4: Nest bay with loaded flow cells



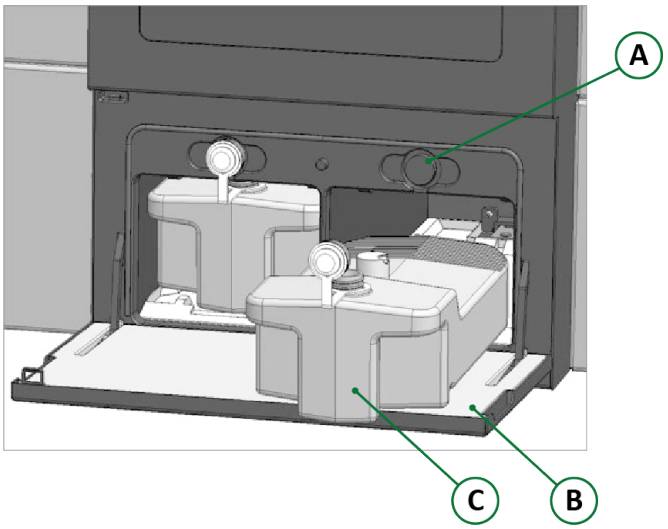
- A Wall around the loading area
- B Loaded flow cell
- C Lid secured over a loaded flow cell
- D Tab to close the lid
- E Button to open the lid
- F Nest status light

Waste Bay

The waste bay holds two waste bottles, one for each side. Two threaded cap holders above the waste bay secure the transport cap tethered to each waste bottle to keep the caps clear of the door.

A sealed tray built into the bottom of the waste bay collects spills and leaks for cleaning and directs liquid to the front of the instrument. Welding prevents liquid from entering the area behind the waste bay. During run or wash setup, a sensor confirms the waste bottle is present and empty and allows the run or wash to proceed. Another sensor detects any spills.

Figure 5: Open waste bay with waste bottles

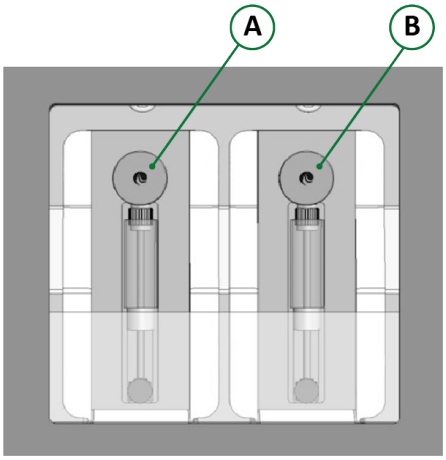


A Cap holder
B Open waste bay door
C Waste bottle

Pump Bays

Each pump bay contains two pumps that control the flow of liquid. The left pump pulls fluid through the left lane of the flow cell and the right pump pulls fluid through the right lane. Keep the pump bay doors, which allow service access, closed during normal operation and maintenance.

Figure 6: Pumps in a pump bay



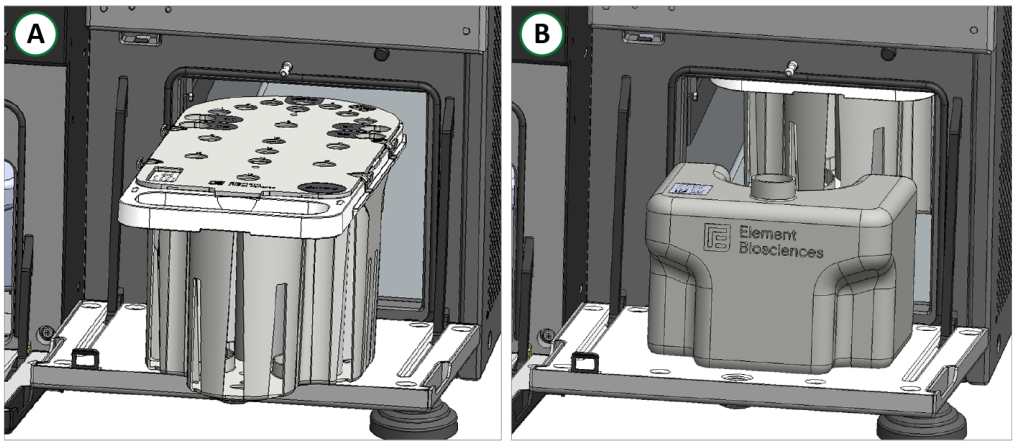
A Pump controlling the left lane of a flow cell
B Pump controlling the right lane of a flow cell

Reagent Bays

Each reagent bay holds a buffer bottle and sequencing basket that contains a cartridge or a wash tray, depending on whether the system is sequencing or washing. Keep the reagent bay doors closed to maintain the refrigeration, which chills reagents.

When priming starts, sippers descend into the bay, pierce the foil seals covering the cartridge wells, and aspirate reagents from the bottom of each well. The sippers continue to aspirate reagents throughout the run. Functioning similarly for a wash, the sippers aspirate wash solution instead of reagents.

Figure 7: Open reagent bay with reagents



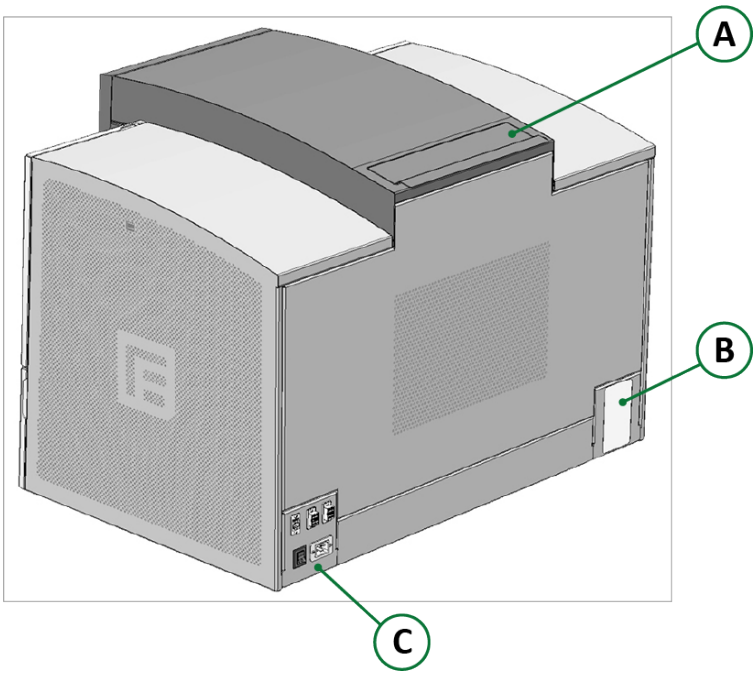
A Loading a basket and cartridge into the reagent bay

B Loading a buffer bottle behind the basket

Back Panel

The back panel includes the air filter tray and input and output (IO) panel. A compliance label displays regulatory symbols for regulatory compliance, the instrument serial number, and electrical specifications. For more information on labeling, compliance, declarations, and certifications, see *Safety and Compliance* on page 90.

Figure 8: Back panel of the instrument



A Air filter tray
B Compliance label
C IO panel

Air Filter Tray

Air enters the instrument through a disposable air filter constructed of pleated paper. The air filter is rated MERV 9, which keeps dust out of the instrument but does not filter smoke or particles < 3 microns. Keeping aerosol and particulate sources away from the instrument extends filter life.

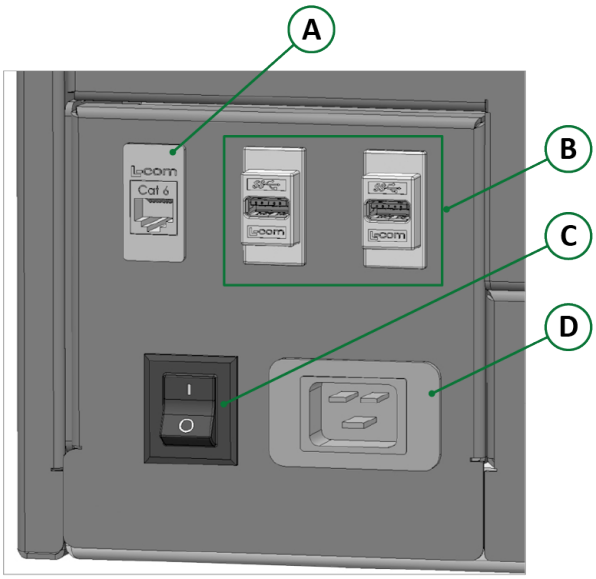
A tray that lifts out of the top of the instrument contains the air filter and facilitates easy replacement. For instructions, see *Replace the Air Filter* on page 67.

Input and Output Panel

An IO panel on the back of the instrument groups connections and the power switch. A Category 6 (Cat6) Ethernet port connects an Ethernet cable and a power entry module connects the power cord. When connecting the instrument to power, use only the power cord that shipped with the instrument.

The IO panel also includes two USB 3.0 ports to connect a mouse, keyboard, or drive for transferring files. Side B includes a third USB 3.0 port. A USB drive that transfer files to or from the instrument must be in ***FAT32 format***.

Figure 9: Power and Ethernet on the IO panel



A Cat6 Ethernet port
B USB 3.0 ports
C Power switch in the on position
D Power entry module

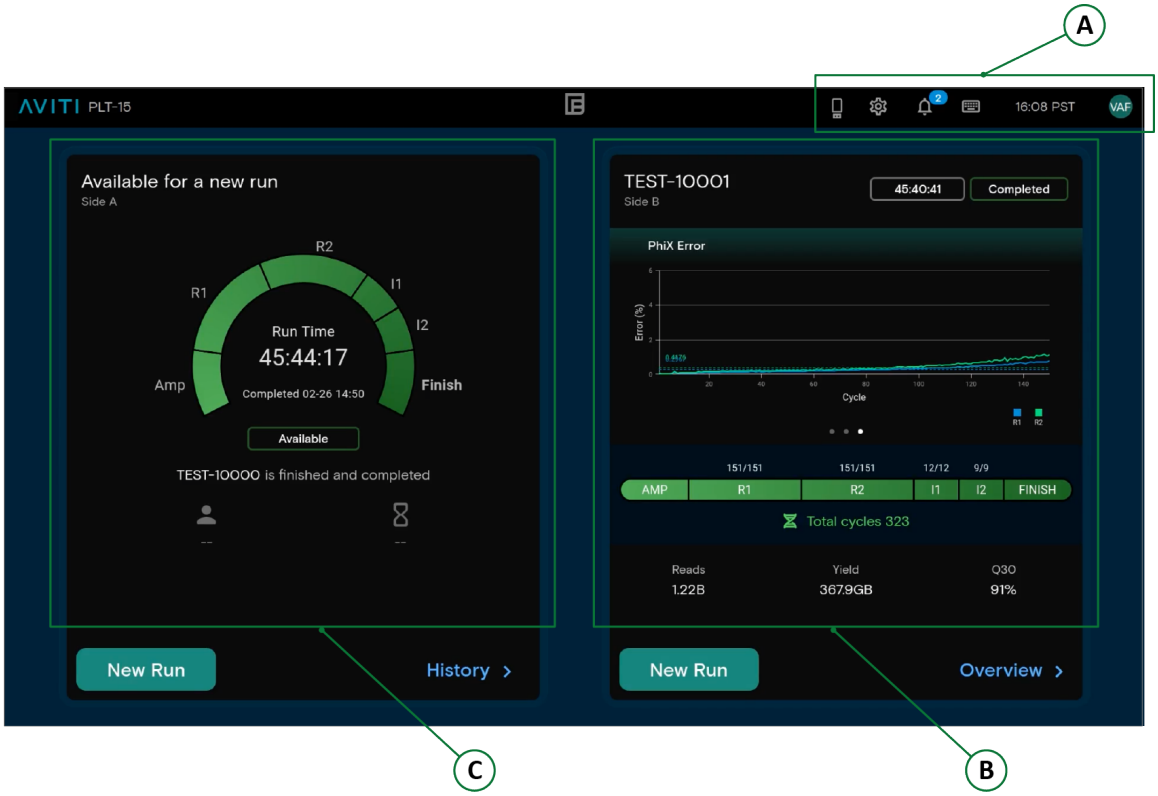
CHAPTER 3

Software and Analysis

AVITI Operating Software

AVITI OS is the main software installed on the AVITI System. The Home screen functions as a system dashboard, displaying the status of each side with features to start runs and washes and monitor sequencing runs.

Figure 10: Home screen components



A Taskbar
B Details view
C Overview view

Home Screen Views

The Home screen includes the following views:

- **Overview**—Displays a general status and past sequencing run time for the side or summarizes an active run or wash.
- **Details**—Displays metrics for an active run. When a run starts, AVITI OS automatically switches to this view.
- **History**—Preserves metrics from the last run. When no run or wash is active, this view is available.

The Overview, Details, and History buttons update the Home screen view. You can switch between details and overview or history and overview.

Taskbar Icons

A taskbar at the top of the Home screen provides the following icons. The Settings and Notifications icons each open a unique workspace. Keyboard, USB Drive, and User access additional features and functions.

Icon	Name	Function
	USB Drive	View a list of USB drives connected to the instrument and safely disconnect a USB drive from the instrument.
	Settings	View system information and configure settings. See *Settings* on page 21.
	Notifications	Review run, wash, instrument, or software notifications and take the indicated action. See *Notifications* on page 22.
	Keyboard	Open a keyboard that accepts touchscreen input.
	User	Open the User menu. Alternatively, this icon displays initials.

Run Start Options

AVITI OS includes the following options for starting a run:

- **Single start**—Set up and start a run on one side of the instrument.
- **Dual start**—Concurrently set up and start runs on both sides of the instrument.
- **Flexible start**—Set up and start a run on one side of the instrument. While the run is in progress, set up and start a run on the other side so sequencing proceeds asynchronously.

AVITI OS allows sequencing with a version 1 kit on one side and a Cloudbreak kit on the other. Because the sides share a camera, this setup increases the duration of the Cloudbreak run.

Run Setup Screens

When you initiate a run, AVITI OS guides you through a series of run setup screens. Each screen indicates how far along setup is and displays callouts to guide consumable loading. Workflow-specific checkboxes ensure the readiness of each consumable.

AVITI OS unlocks the reagent and waste bay doors at the appropriate steps. Closing a door validates consumable or accessory presence and scans consumable barcodes. After the waste step, priming starts automatically. Priming prepares reagents for delivery and pumps air and reagents through a used flow cell and the fluidic tubes, preventing contamination between runs.

Wash Setup Screens

Initiating a wash opens a series of wash setup screens that guide you through setting up a maintenance, standby, or recovery wash. Wash setup functions similar to run setup, but closing the door validates the wash tray presence.

Flexible Start

Flexible start safely pauses the active run and initiates a sequencing run on the other side of the instrument. When setting up the second run, AVITI OS finds a safe pause point before proceeding. Pausing the first run typically takes several minutes but can take as long as ~2 hours, depending on the run stage.

When you initiate flexible start, AVITI OS displays the expected wait time. AVITI OS also includes options to cancel flexible start and resume the active run.

Settings

Settings includes configurable and read-only settings that control the instrument profile and system connections. AVITI OS divides the settings among the following tabs. The Network and Storage tabs include connectivity indicators. The User tab is unique to offline systems and online systems with local authentication.

- **About**—Displays software and instrument information:
  - AVITI OS version and the last license acceptance date
  - AVITI System name, serial number, available local storage, and compute ID
  - Updates available for system firmware and software
- **General**—Controls the system name, displays telemetry and elevation settings, and exports log files from offline systems.
- **Network**—Controls network and internet connections for the system.
- **Storage**—Lists storage connections with settings for adding and managing storage connections.
- **User**—Provides password management for offline systems and online systems with local authentication.

NOTE
Compute ID is a unique code for the integrated circuit that identifies the instrument computer.

Network Status

The Network tab displays the following icons, which indicate the status of the network connection. An additional Indicator appears on the tab to show internet connectivity.

Icon	Network Status
	Connected
	Local internet only
	Disconnected

Storage Status

The Storage tab displays the following icons, which indicate the status of the storage connections. An additional Indicator appears on the tab to show storage connectivity.

Icon	Storage Status
	At least one storage connection
	No storage connection

Notifications

Notifications display system messages across three tabs: General, Side A, and Side B. Expand a notification to see the message, date, and time.

Table 2: Types of notifications

Notification	Icon	Description	Action
Success		A run or wash completed successfully.	Acknowledge successful completion.
Information		The software is ready to be updated to a new version.	Acknowledge the update.
Warning		The system requires your attention, but you can continue operation.	Acknowledge the warning and resolve it by the indicated date.
Error		The system has malfunctioned and requires action to proceed.	Follow the onscreen prompt.

Unread Notifications

Notifications include badges that indicate the number of unread messages. Checkboxes mark notifications as read or unread. Marking a notification as read can reset the status lights on that side of the instrument.

Icon	Name	Action
	Mark as read	Mark the selected notifications as read.
	Mark as unread	Mark the selected notifications as unread.

Filtering and Sorting

Notifications include filters with sorting from newest to oldest or oldest to newest.

Filter	Description
All	View all messages on the selected tab.
Read	View only read messages on the selected tab.
Unread	View only unread messages on the selected tab.

Signing In and Out

Signing in to AVITI OS requires the email address and password for your organization. The first time you sign in to AVITI OS after instrument installation or an update, you must accept the license agreement. A Logout option on the User menu signs you out.

If requested, Element can enable local authentication mode for an online system. This feature assigns a fixed user name and user-defined password to sign in.

Primary Analysis

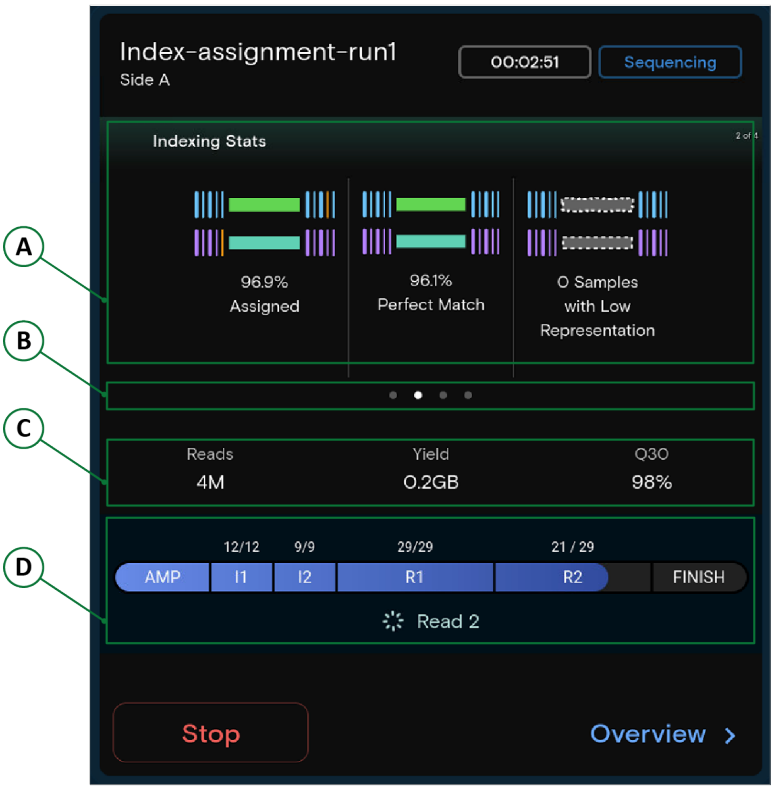
Onboard primary analysis software calls bases, assigns quality scores (Q-scores), and generates run metrics. The software extracts and corrects intensities from images to call a base, then assigns a Q-score to the base to indicate confidence in the call.

Run Monitoring

During a run, the Details view displays primary analysis-generated run metrics that monitor overall run health and progress. As the run progresses, metrics appear followed by regular updates. The metrics are included in the run output and remain onscreen until you set up a new sequencing run.

Run metrics are divided into Reads, Yield, and Q30 fields and a series of charts that you can cycle through: % Q30, Indexing Stats, PhiX Error, and Run Configuration. PhiX Error applies only to runs that include a spike in of PhiX Control Library. Indexing Stats apply only to runs that include a run manifest with 1–364 index pairs.

Figure 11: Details view for a run on side A



A Indexing Stats chart
B Navigation to additional run metrics charts
C Run metrics fields
D Run progress and cycles for each stage

Run Metrics

The following table lists the run metrics displayed on the Home screen. Taken together, metrics on the Indexing Assignment tab estimate index quality and provide an early alert to potential problems with the run manifest or library.

Metric	Tab	Description
Reads	Not applicable	The number of reads the run is generating.
Yield	Not applicable	The amount of sequencing data in gigabases the run is expected to produce.
Q30	Not applicable	The percentage of base calls ≥ Q30. Q30 indicates a 1 in 1000 possibility of an incorrect base.
% Q30	Q30	A chart indicating the percentage of base calls in each Read 1 and Read 2 cycle that have a Q-score ≥ Q30.
Assigned	Indexing Assignment	The percentage of reads with indexes assigned.
Perfect Match	Indexing Assignment	The percentage of reads with indexes assigned that have a perfect index match.
Samples with Low Representation	Indexing Assignment	The number of samples with < 10,000 polonies assigned.
PhiX error rate	PhiX Error Rate	The percentage of control reads in each Read 1 and Read 2 cycle that do not align to PhiX Control Library.

Run Output and Storage

The output of a run is the run folder, which contains bases files and other run data. A storage connection transfers the run folder from the instrument to your storage location, which can be local or in the cloud. For more information, see *Storage Connections* on page 69.

After a run, use Bases2Fastq Software to perform demultiplexing and convert the bases files into FASTQ files for secondary analysis with third-party software of your choice. Bases files contain genomic data and are the primary output of a run.

Figure 12: Data processing components

Run Folder

A run folder is named for the run name and contains the following files, which are organized at the root directory or in a subfolder. Braces around a file name component indicate variability. For example, the value for {tile} depends on the applicable tile number.

File	Directory and File Name	Description	Quantity
Alignment	Root/Alignment/{read}_{tile}.aln	Binary files that indicate which polonies align to PhiX Control Library	One per tile per read
Bases	Root/BaseCalls/{tile}/{read}_{tileName}_C{cycle:000}.bases.gz	Binary files that contain base calls and associated Q-scores	One per read, tile, and cycle
Filter	Root/Filter/{tile}.filter	Binary files that contain the filter status for each polony	One per tile
Index assignment	Root/IndexAssignment.csv	Comma-separated values (CSV) file that records the results of onboard demultiplexing	One per run
Location	Root/Location/{tile}.loc	Binary files that identify polony locations on the flow cell	One per tile
Run manifest	Root/RunManifest.csv	CSV file that records biological sample information and analysis settings	One per run
	Root/RunManifest.json	JavaScript Object Notation (JSON) file reserved for Element processes	One per run
Run parameters	Root/RunParameters.json	JSON file that records information about the run configuration	One per run
Run statistics	Stats/AvitiRunStats.json	JSON file that records run metrics	One per run

File	Directory and File Name	Description	Quantity
Run uploaded	Root/RunUploaded.json	Empty JSON file that is the last file transferred and marks run completion	One per run

Run Manifest

The run manifest stores the information for the contents of a sequencing run and analyzing the results. The run manifest includes demultiplexing settings, FASTQ file settings, and a list of samples with any corresponding index sequences.

Sequencing indexed libraries requires preparing a run manifest for the run. When a run does not include a run manifest, AVITI OS generates a default run manifest that assigns all reads to one sample during FASTQ generation. Thus, demultiplexing indexed libraries with a default run manifest is ***not possible***. For help preparing a run manifest, see the *Run Manifest Workflow Guide (MA-00011)*.

Local Disk Storage

Because the system software transfers runs to off-instrument storage locations, local disk storage is intended only for temporary storage. Accordingly, the instrument hard drive has sufficient space to store two runs and start an additional two runs.

When you initiate run setup, AVITI OS checks whether the system has sufficient space to support the run. If AVITI OS indicates that the system does not have sufficient space, contact Element Technical Support.

Telemetry

Separate from the transfer of genomic data to your storage location, ***which Element cannot access***, telemetry sends instrument health data to Element. These data help support maintenance and troubleshooting and do not include any confidential information.

Telemetry is limited to the following data:

- **Software metrics**—Software and firmware versions, CPU and memory metrics, and the instrument serial number, ID, and name. These data are communicated as part of regular telemetry events.
- **Hardware metrics**—Data on motors, fans, lasers, and other instrument hardware, which helps Element understand the probable condition of select hardware components.
- **System logs**—Routine logs the system generates when idle or running. The logs include power cycle times, errors, internal communications, and the status of internal services.
- **Primary analysis metrics**—Sequencing metrics, including data for Q30 scores, error rates, and index assignment metrics. The index assignment data exclude sample names.
- **Run information**—Data communicated for a run, including run name and ID, run side, run start and end dates and times, run type (sequencing or washing), consumable information, and the number of cycles per read. The data exclude run descriptions.
- **Run logs**—Run-specific information from a subset of system logs. Data include recipe execution, the timing of run steps, and communications between software, firmware, and hardware.

CHAPTER 4

Sequencing Materials

Sequencing Kits

A sequencing kit provides the flow cell and reagents for a run. Performing a run on one side of the instrument requires one sequencing kit in a 2 x 75 or 2 x 150 configuration. A dual or flexible start run requires two kits. To ensure the compatibility of run components, see *System Compatibility* on page 6.

When using a sequencing kit and other reagents, always wear personal protective equipment (PPE): a lab coat, powder-free disposable gloves, and protective goggles. Review the safety data sheets (SDS) for chemical properties. The SDS inform safety, disposal, and hazards for your region and are available at go.elembio.link/sds.

Sequencing Kit Contents and Storage

Each sequencing kit is single-use and packaged in two boxes. The following tables list the kit contents and storage requirements. When you receive your kit, promptly store the components at the proper temperatures.

CAUTION
The cartridge contains light-sensitive reagents. Keep the cartridge packaged until use and ***protect from light***.

AVITI 2x75 Sequencing Kit

Component	Quantity	Shipping Temperature	Storage Temperature
AVITI 2x75 Sequencing Cartridge	1	-25°C to -15°C	-25°C to -15°C
AVITI Flow Cell Cartridge	1	Room temperature	2°C to 8°C
AVITI Oligonucleotide Set	1	-25°C to -15°C	-25°C to -15°C
AVITI Universal Wash Buffer	1	Room temperature	Room temperature
Library Loading Buffer	2	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit

Component	Quantity	Shipping Temperature	Storage Temperature
AVITI 2x150 Sequencing Cartridge	1	-25°C to -15°C	-25°C to -15°C
AVITI Flow Cell Cartridge	1	Room temperature	2°C to 8°C
AVITI Oligonucleotide Set	1	-25°C to -15°C	-25°C to -15°C
AVITI Universal Wash Buffer	1	Room temperature	Room temperature
Library Loading Buffer	2	-25°C to -15°C	-25°C to -15°C

AVITI 2x75 Sequencing Kit Cloudbreak

Component	Quantity	Shipping Temperature	Storage Temperature
AVITI 2x75 Cartridge Cloudbreak	1	-25°C to -15°C	-25°C to -15°C
AVITI Flow Cell Cloudbreak	1	Room temperature	2°C to 8°C
Adept Primer Set Cloudbreak	1	-25°C to -15°C	-25°C to -15°C
AVITI Universal Wash Buffer	1	Room temperature	Room temperature
Library Loading Buffer	2	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit Cloudbreak

Component	Quantity	Shipping Temperature	Storage Temperature
AVITI 2x150 Cartridge Cloudbreak	1	-25°C to -15°C	-25°C to -15°C
AVITI Flow Cell Cloudbreak	1	Room temperature	2°C to 8°C
Adept Primer Set Cloudbreak	1	-25°C to -15°C	-25°C to -15°C
AVITI Universal Wash Buffer	1	Room temperature	Room temperature
Library Loading Buffer	2	-25°C to -15°C	-25°C to -15°C

Kit Components

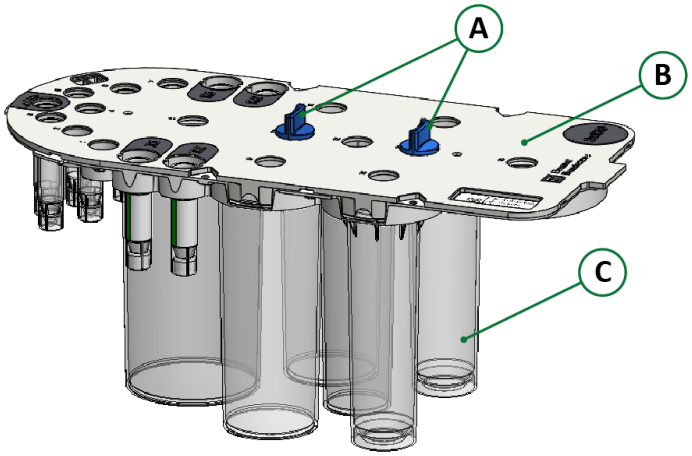
The components in a 2 x 75 and 2 x 150 kit are the same except for the sequencing cartridge. A 2 x 150 cartridge includes a greater volume of reagents to support more cycles. For more information, see *Number of Cycles* on page 9.

Each kit component includes a barcode label for tracking and validation purposes.

Sequencing Cartridge

A sequencing cartridge packages the amplification, paired-end, indexing, and cycling reagents and a post-run wash buffer into a convenient container that facilitates reagent preparation, loading, and disposal. A lid covers the cartridge to retain and label the reagents. Two shipping locks secure the lid and remain intact until run setup.

Each reagent occupies a foil-sealed well that is transparent to allow visual inspection. The Library well is reserved for diluted sequencing library. The I1, I2, R1, and R2 wells contain replaceable primers. Some wells are intentionally empty. For a cartridge map, see *Discard the Cartridge and Bottle* on page 55.

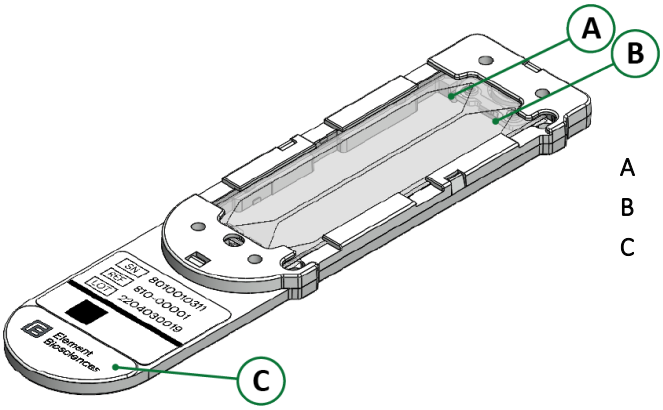


A Shipping locks
B Cartridge lid
C Transparent well

Flow Cell

The flow cell is a two-lane glass substrate encased in a plastic cartridge. The outlet end of the cartridge extends into a handle with a gripper for safe handling.

Proprietary surface chemistry coats the flow cell and enables polony generation and sequencing. Library and reagents enter the flow cell through inlet ports at the top of each lane, saturating the surface and exiting as waste through outlet ports at the bottom.



A Lane 1
B Lane 2
C Gripper

Adept Primer Set

The primers in the I1, I2, R1, and R2 wells of the cartridge support Elevate libraries. Sequencing Adept libraries requires replacing the prepackaged primers with the tubes from the AVITI Oligonucleotide Set or Adept Primer Set Cloudbreak.

Table 3: Primer set contents

Primer	AVITI Oligonucleotide Set	Adept Primer Set Cloudbreak
Index 1	Adept Index 1 (I1) Primer	Adept Index 1 (I1) Primer Cloudbreak

Primer	AVITI Oligonucleotide Set	Adept Primer Set Cloudbreak
Index 2	Adept Index 2 (I2) Primer	Adept Index 2 (I2) Primer Cloudbreak
Read 1	Adept Read 1 (R1) Primer	Adept Read 1 (R1) Primer Cloudbreak
Read 2	Adept Read 2 (R2) Primer	Adept Read 2 (R2) Primer Cloudbreak

Loading and Wash Buffers

A sequencing kit includes multiple loading and wash buffers. The loading and wash buffers are packaged separately. Instrument Wash is included in the cartridge.

Table 4: Buffer types

Buffer	Packaging	Description
Library Loading Buffer	Tube	Dilutes the experimental library and optional PhiX Control Library to the target loading concentration before a run
AVITI Universal Wash Buffer	Buffer bottle	Flushes excess reagents from the flow cell between certain chemistry steps
Instrument Wash	Cartridge	Serves as the wash solution for the automatic post-run wash

Controls and Custom Primers

In addition to the sequencing kits, Element offers PhiX Control Library and custom primer sets. These components supplement the sequencing kits, providing additional reagents for runs that include sequencing controls and custom primers.

PhiX Control Library

PhiX Control Library is a color-balanced, ready-to-use library that functions as a positive control and adds diversity to low-complexity libraries. Each type of PhiX Control Library includes unique index sequences, which the *Run Manifest Workflow Guide (MA-00011)* lists.

Table 5: Control library types

Type	Format	Stock Concentration	Shipping and Storage Temperature
PhiX Control Library, Adept	Circular	1 nM	-25°C to -15°C
PhiX Control Library, Elevate	Circular	1 nM	-25°C to -15°C
Cloudbreak PhiX Control Library, Elevate	Linear	1 nM	-25°C to -15°C

Custom Primer Sets

A custom primer set provides read-specific buffers for preparing custom primers to sequence Adept libraries. The buffers are packaged in tubes that fit the I1, I2, R1, and R2 wells of the sequencing cartridge.

Table 6: Custom primer set contents

Custom Primer Set	Buffers	Shipping and Storage Temperature
Adept Custom Oligonucleotide Buffer Set	Adept Custom Index 1 (I1) Buffer	-25°C to -15°C
	Adept Custom Index 2 (I2) Buffer	-25°C to -15°C
	Adept Custom Read 1 (R1) Buffer	-25°C to -15°C
	Adept Custom Read 2 (R2) Buffer	-25°C to -15°C
Adept Custom Primer Set Cloudbreak	Adept Custom Index 1 Buffer, Index First (I1)	-25°C to -15°C
	Adept Custom Index 2 Buffer, Index First (I2)	-25°C to -15°C
	Adept Custom Read 1 Buffer, Index First (R1)	-25°C to -15°C
	Adept Custom Read 2 Buffer, Index First (R2)	-25°C to -15°C

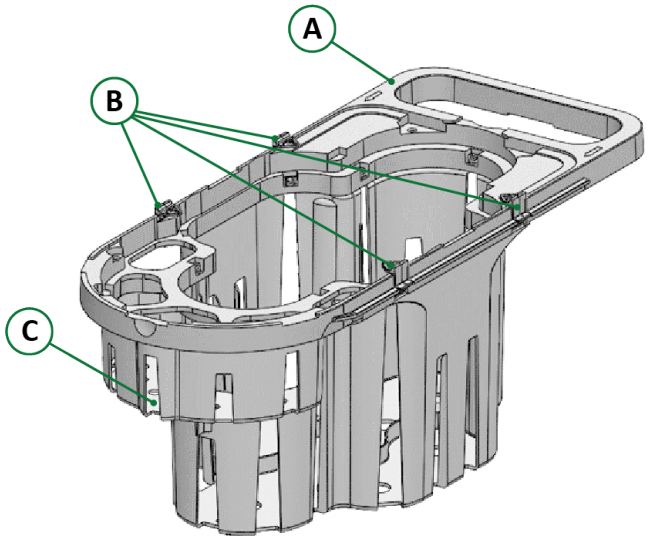
Reusable Accessories

Sequencing baskets, wash trays, and waste bottles support run setup and washes while minimizing waste. These accessories are reusable but require periodic replacement.

Sequencing Basket

A sequencing basket protects the cartridge during a run. The back of the basket extends into a handle with arrows that indicate the loading direction. Clips along the top of the basket secure the cartridge. The curved area under the handle accommodates the buffer bottle, which is loaded into the reagent bay behind the basket. A window at the front of the basket enables library inspection.

Figure 13: Sequencing basket features



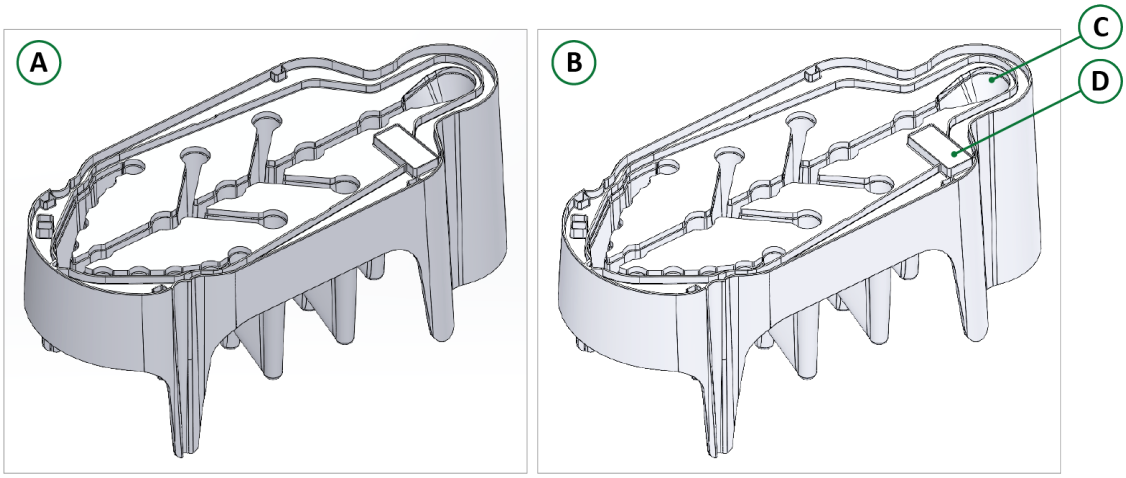
A Handle at the back
B Latches along the top
C Window at the front

Wash Trays

The AVITI System includes two types of wash trays, each dedicated to different wash solutions:

- AVITI Wash Tray 1, Gray, for use with Wash 1 Solution.
- AVITI Wash Tray 2, White, for use with Wash 2 Solution and nuclease-free water.

The back of a wash tray forms a handle with a fill area for adding wash solution. Interior fill lines indicate approximate volumes and an overflow wall contains any wash solution that exceeds the 800 ml maximum fill volume. Each tray includes a water-proof barcode label for validation purposes and a clear lid to prevent spills during transportation.

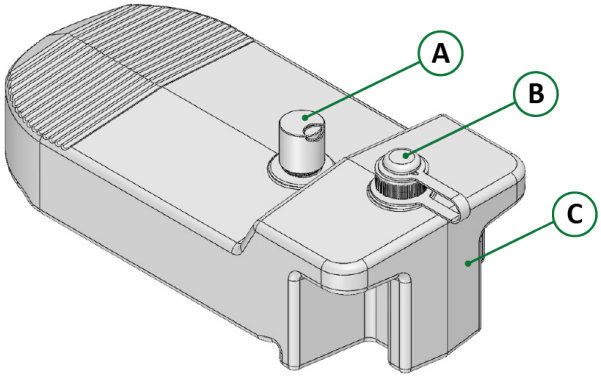


A Gray wash tray
B White wash tray
C Handle with fill area
D Barcode label

Waste Bottle

A waste bottle collects spent reagents and library. The maximum capacity of 3.2 L per bottle is sufficient to contain all waste from one run on one side of the instrument. Attaching a funnel to the waste receptacle prevents splashing and spills when emptying waste, particularly for smaller receptacles.

Two caps on top of the waste bottle contain waste: a transport cap and a vent cap. The transport cap is tethered and sits above the handle, sealing the bottle for transportation of waste. The vent cap sits lower on the bottle. Opening the vent cap when emptying waste improves the flow of liquid. Ridges on the back of the bottle and a handle at the front facilitate handling.



A Vent cap
B Transport cap
C Handle

User-Supplied Materials

Instrument operation and maintenance use the materials listed in the following tables. To ensure compatibility of Element run components, see *System Compatibility* on page 6.

Library prep requires a separate set of materials, which are listed in the Adept and Elevate workflow guides and materials lists.

User-Supplied Sequencing Consumables

Supplier	Consumable	Catalog #
General lab supplier	0.2 M Tris-HCl, pH 7.0	Not applicable
	1 N NaOH	Not applicable
	10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA	Not applicable
	Filtered pipette tips	Not applicable
	Low TE buffer	Not applicable
	Nuclease-free water	Not applicable
Element Biosciences	Any sequencing kit: • AVITI 2x75 Sequencing Kit • AVITI 2x150 Sequencing Kit • AVITI 2x75 Sequencing Kit Cloudbreak • AVITI 2x150 Sequencing Kit Cloudbreak	The corresponding catalog #: • 860-00002 • 860-00001 • 860-00004 • 860-00003
	[Optional] Either custom primer set: • Adept Custom Oligonucleotide Buffer Set • Adept Custom Primer Set Cloudbreak	The corresponding catalog #: • Catalog # 820-00008 • Catalog # 820-00009
	[Optional] Any control library: • PhiX Control Library, Adept • PhiX Control Library, Elevate • Cloudbreak PhiX Control Library, Elevate	The corresponding catalog #: • Catalog # 830-00004 • Catalog # 830-00002 • Catalog # 830-00017
Eppendorf	DNA LoBind Tubes, 2 ml	Catalog # 022431048

User-Supplied Maintenance Consumables

Supplier	Consumable	Catalog #
General lab supplier	Microfiber cloths	Not applicable
	Nuclease-free water	Not applicable
	Polyurethane foam-tip swabs with plastic handles	Not applicable
	Serological pipettes	Not applicable
	Simple Green All-Purpose Cleaner	Not applicable*
Element Biosciences	Air Filter, MERV 9	Catalog # 350-00065
MilliporeSigma	Sodium hypochlorite solution, reagent grade, 4.00–4.99%, 500 ml	Catalog # 239305*
Thermo Fisher Scientific	Either bottle: • Nalgene HDPE Heavy-Duty Bottles with Closure, 2 L • Nalgene Large Narrow-Mouth LDPE Bottles, 2 L	The corresponding catalog #: • 2125-2000PK* • 2202-0005PK*
VWR	Alcohol Prep Pads, 70% isopropyl alcohol	Catalog # 95041-712*
	Tween 20, reagent grade, 1 L	Catalog # 97062-332*

* Or equivalent

User-Supplied Equipment and Accessories

Supplier	Consumable	Catalog #
General lab supplier	Freezer, -25°C to -15°C	Not applicable
	Ice bucket	Not applicable
	Mini centrifuge	Not applicable
	Pipette controller	Not applicable
	Pipettes, single-channel	Not applicable
	Refrigerator, 2°C to 8°C	Not applicable
	Tub for water baths	Not applicable
	Vortex mixer	Not applicable
	[Optional] FAT32 USB drive	Not applicable
Element Biosciences	AVITI Sequencing Basket	Catalog # 890-00025
	AVITI Wash Tray 1, Gray	Catalog # 890-00023
	AVITI Wash Tray 2, White	Catalog # 890-00027
	AVITI Waste Bottle	Catalog # 890-00024
Uline	[Optional] Heavy-Duty Funnel, 64 oz	Model # H-5216

CHAPTER 5

Consumable Prep

Input Recommendations

The recommended input for sequencing is ≥ 1 nM library. The input library is normalized to 1 nM, denatured into single strands, and diluted to the target loading concentration. When starting with a 0.2–1 nM library, the library is denatured and diluted but not normalized. Libraries < 0.2 nM are not supported.

PhiX Control Library Spike-In

For most applications, Element recommends a spike-in of PhiX Control Library. The following spike-in percentages optimize the benefits of PhiX Control Library for specific experiments.

Table 7: Spike-in recommendations

Experiment	Spike-In (%)
QC and error rate reporting	> 2
Low-diversity libraries*	≥ 5
Low-complexity indexing (≥ 2-plex)	> 2

* The first five cycles of Read 1 require high diversity.

Loading Concentration

The optimal loading concentration achieves a total polony count that ranges from ~800 million to 1 billion reads. The polony count increases as the loading concentration increases, which can compromise data quality. Low polony counts promote high data quality but lower the amount of data output.

The following loading concentrations target the total polony count range and provide a starting point for determining your optimal loading concentration. Some libraries require a loading concentration that is higher or lower than the indicated ranges. If you are sequencing pooled libraries, the pool must contain libraries with similar size distributions.

Table 8: Target loading concentrations

Library Size (bp)*	Adept Loading Concentration (pM)	Elevate Loading Concentration (pM)
Small (150–350)	5–7	5–9
Medium (350–600)	7–11	7–10
Large (≥ 600)	11–15	12–18

* Library size is the full length of the library, including the DNA insert, adapters, and primers.

Custom Primers

The AVITI System supports any combination of I1, I2, R1, and R2 custom primers to sequence Adept libraries. The custom primers must be HPLC-purified and prepared using the applicable method:

- **Spike-in**—Spike custom primers into the primer tubes provided in the AVITI Oligonucleotide Set or Adept Primer Set Cloudbreak.
- **Replacement**—Replace the primers in the cartridge with buffer tubes from the Adept Custom Oligonucleotide Buffer Set or Adept Custom Primer Set Cloudbreak and add custom primers.

Element oligonucleotides include sequencing primers that are only compatible with standard Nextera and TruSeq libraries. Adept libraries with sequencing primer binding sites that are not standard Nextera or TruSeq require custom primers.

Custom primers require special consideration and planning. To make sure a run that includes custom primers meets specifications, contact Element Technical Support early in experiment planning. Element Technical Support can also help determine whether your library requires custom primers and which preparation method applies.

Prepare the Cartridge

Preparing the sequencing cartridge adds Adept primer tubes as needed and thaws reagents. The subsequent dilution procedure includes the option to store a normalized library. If you intend to store, do not prepare the cartridge until you are ready to sequence. Prepare the cartridge within a day or two of sequencing.

CAUTION
The cartridge contains light-sensitive reagents. ***Protect the cartridge from light*** until loading onto the instrument. Additionally, do not remove the cartridge lid.

Add Adept Primer Tubes

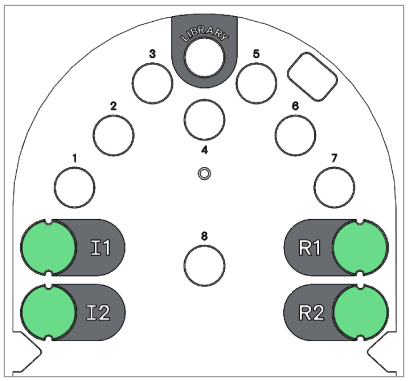
1. If you are sequencing Elevate libraries, skip the following steps and proceed to *Thaw the Cartridge* on page 44.

   —The sequencing cartridge contains Elevate primers, so only Adept libraries require primer replacement.—
2. Remove a cartridge and primer set from -25°C to -15°C storage. Reference the following table to determine the applicable primer set.

Primer Strategy	Primer Set
No custom primers	AVITI Oligonucleotide Set or Adept Primer Set Cloudbreak
Custom primers (spike-in method)	AVITI Oligonucleotide Set or Adept Primer Set Cloudbreak
Custom primers (replacement method)	Adept Custom Oligonucleotide Buffer Set or Adept Custom Primer Set Cloudbreak

   —Subsequent procedures prepare custom primers and load them into the cartridge.—
3. Twist the primer tubes in wells I1, I2, R1, and R2 left to unlock.

   Figure 14: Primer tube wells



4. Remove the primer tubes from the cartridge and discard per the SDS.
5. Insert the tubes from the primer set into the vacated wells. Match the abbreviation on the tube label to the well label.

   —For example, insert Adept Custom Index 1 (**I1**) Buffer Cloudbreak into the **I1** well.—
6. Twist each tube right until it locks into place.

Thaw the Cartridge

Use a water bath (recommended) ***or*** refrigerator to thaw reagents in the cartridge. Steps to mix reagents and load diluted sequencing library into the cartridge occur during run setup.

Thaw Reagents in a Water Bath

1. If necessary, remove a cartridge from -25°C to -15°C storage.
2. Prepare a room-temperature water bath.
3. Place the cartridge in the water bath. Do not submerge.
4. Thaw the cartridge for the applicable duration:
   - » For a 2 x 75 cartridge, thaw for ~90 minutes. Place an object onto the cartridge to prevent floating and tilting.
   - » For a 2 x 150 cartridge, thaw for ~2.5 hours.
5. While the cartridge thaws, denature and dilute the library. See *Dilute Library and Custom Primers* on page 45.
6. Inspect each well of the cartridge wells to make sure reagents are fully thawed.
7. If any ice remains, return the cartridge to the water bath until fully thawed.
8. Set aside the thawed cartridge at room temperature. If you are not sequencing immediately, keep the cartridge at 2°C to 8°C.

Thaw Reagents in a Refrigerator

1. If necessary, remove a cartridge from -25°C to -15°C storage.
2. Place the cartridge in a 2°C to 8°C refrigerator.
3. Thaw the cartridge for the applicable duration:
   - » For a 2 x 75 cartridge, thaw for ~8 hours.
   - » For a 2 x 150 cartridge, thaw for ~24 hours.
4. Inspect each well of the cartridge wells to make sure reagents are fully thawed.
5. If any ice remains, continue thawing:
   a. Place the cartridge in a room-temperature water bath. Do not submerge.
   b. Thaw the cartridge for the applicable duration:
      - For a 2 x 75 cartridge, thaw for ≤ 1 hour. Place an object onto the cartridge to prevent floating and tilting.
      - For a 2 x 150 cartridge, thaw for ≤ 20 minutes.
   c. While the cartridge thaws, denature and dilute the library. See *Dilute Library and Custom Primers* on page 45.
6. Set aside the thawed cartridge at room temperature. If you are not sequencing immediately, keep the cartridge at 2°C to 8°C.

Dilute Library and Custom Primers

The library dilution procedures prepare 1.4 ml diluted sequencing library at the target loading concentration with an optional spike-in. Custom primers are also diluted as applicable.

Prepare the Library

1. Gather the following consumables:
   - » 0.2 M Tris-HCl buffer, pH 7.0
   - » 1 N NaOH
   - » 2 ml DNA LoBind tubes (4–7)
   - » 10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA (low TE buffer)
   - » Nuclease-free water
2. Combine the following reagents to prepare 0.2 N NaOH. Use 0.2 N NaOH within the day and discard.

Reagent	Volume (µl)
1 N NaOH	20
Nuclease-free water	80
Total	100

3. Remove the following components from -25°C to -15°C storage and thaw on ice:
   - » Library Loading Buffer
   - » Experimental library
   - » [Optional] PhiX Control Library
4. Pulse vortex the thawed libraries and briefly centrifuge.
5. If the experimental library is ≥ 1 nM, normalize:
   a. In a new DNA LoBind tube, use low TE buffer to dilute the library to 1 nM.
   b. Proceed immediately or cap the tube, store the 1 nM library at -25°C to -15°, and sequence within the allotted time.

—Adept and Elevate v1 libraries require sequencing within 15 days of circularization.—

Denature the Library with NaOH

1. Calculate the loading concentration of each library, experimental and control, based on the target loading concentration and relative amount of each library:

$$loading\ concentration\ in\ pM = target\ loading\ concentration\ in\ pM * library\ amount\ in\ \%$$

—For example, if the target loading concentration is 9 pM with a 2% spike-in: the experimental library concentration is 8.82 pM (9 pM * 98%) and the control library concentration is 0.18 pM (9 pM * 2%).—

NOTE
The experimental and control library concentrations do not need to match.

2. Calculate the experimental library volume based on the calculated loading concentration and a 1.4 ml loading volume:

   $$\textit{library volume in } \mu l = (\textit{library loading concentration in } pM * 1400\ \mu l)/\textit{library starting concentration in } pM$$

   —Continuing the preceding example and assuming a 1 nM starting concentration, the library volume is 12.3 µl: (8.82 pM * 1400 µl)/1000 pM.—

3. If you are adding a spike-in, calculate the control library volume based on the loading concentration and a 1.4 ml loading volume:

   $$\textit{control library volume in } \mu l = (\textit{control library loading concentration in } pM * 1400\ \mu l)/\textit{control library concentration in } pM$$

   —Continuing the preceding example and assuming a 1 nM PhiX Control Library, the control library volume is 0.25 µl: (0.18 pM * 1400 µl)/1000 pM.—

4. If step 3 calculated a volume < 1 µl, dilute PhiX Control Library in low TE buffer to use a volume ≥ 1 µl for accurate pipetting.
5. Record the total volume of diluted sequencing library (experimental and control) in µl.

   —This procedure uses equal volumes of library, 0.2 N NaOH, and 0.2 M Tris-HCl buffer, pH 7.0. Therefore, the volume recorded at this step is used in two subsequent steps.—

6. Combine the library volumes calculated in steps 2 and 3 in a new DNA LoBind tube.
7. Add an equal volume of freshly prepared 0.2 N NaOH.
8. Vortex the tube to mix and briefly centrifuge.
9. Incubate the tube at room temperature for 5 minutes to denature the library.
10. Vortex the tube to mix and briefly centrifuge.
11. Add 0.2 M Tris-HCl buffer, pH 7.0 at an equal volume of 0.2 N NaOH to neutralize the reaction.
12. Vortex the tube to mix and briefly centrifuge.

    —The library is denatured, neutralized, and at 1/3 the input concentration in 3x input volume.—

13. Add a sufficient volume of Library Loading Buffer to reach a total volume of 1.4 ml:

    $$\textit{buffer volume in } \mu l = 1400\ \mu l - 3 * \textit{library volume in } \mu l$$

14. Vortex the tube to mix and briefly centrifuge.
15. Place the diluted sequencing library on ice. Use within the day.

Prepare Custom Primers

1. If you are not using custom primers, skip the following steps and proceed to *Set Up a Run* on page 48.
2. In a new DNA LoBind tube, prepare each applicable custom primer using low TE buffer:

Custom Primer	Volume (µl)	Concentration (µM)
Index 1	19	100
Index 2	19	100
Read 1	32.4	100
Read 2	19	100

3. Set aside the 100 µM custom primers on ice. Use within the day.

CHAPTER 6

Sequencing

Set Up a Run

Setting up a run defines the run parameters and loads sequencing consumables. For help discarding run setup, recovering from errors, and other troubleshooting information, see *Troubleshooting* on page 82.

NOTE
Before priming, you can discard run setup and save the cartridge.

Initiate a Sequencing Run

1. Gather the following materials:
   - » Buffer bottle
   - » Cartridge
   - » Sequencing basket
   - » Towel or wipe
   - » Used flow cell

   —A used flow cell might already be in the instrument.—
2. If applicable, stage run manifests for import:
   a. Save a run manifest to a USB drive or SMB within the path specified for the storage connection.
   b. Repeat step a as needed to stage a second run manifest for a dual start run.
   c. If you saved run manifests to a USB drive, connect the USB drive to a port on the side or back of the instrument.
3. On the Home screen, select **New Run**.
4. If AVITI OS prompts that the flow cell is missing, load a ***used*** flow cell:
   a. Select **Open Nest**.
   b. Place the used flow cell onto the nest and close the lid.
   c. Select **Close Nest**.
5. Select which side to sequence on:
   - » **Side A**—Set up a run on side A.
   - » **Both**—Set up runs on sides A and B.
   - » **Side B**—Set up a run on side B.
6. Select **Sequence**, and then select **Next** to proceed to the Prepare Reagents screen.

Inspect and Mix Reagents

1. Inspect each cartridge well to make sure reagents are fully thawed.
2. Make sure the cartridge contains the appropriate primers.
3. Make sure the tubes in the I1, I2, R1, and R2 wells are secure. If necessary, twist each tube to the left.
4. Gently invert the cartridge ***10 times*** to mix reagents.

   CAUTION
   Inadequately mixed reagents can cause run failure.
5. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.

6. Inspect the small tubes to make sure reagents are settled at the bottom.
7. Place the cartridge into a clean sequencing basket and lock the clips. Wipe any excess moisture.

Add Custom Primers to the Cartridge

1. If you are not using custom primers, skip the following steps and proceed to *Add Library to the Cartridge*.
2. Using a new 1 ml pipette tip, pierce the center of the applicable I1, I2, R1, and R2 wells to create one hole. Push the foil to the edges.
3. Discard the pipette tip.
4. Add the applicable volume of 100 µM custom primer to each pierced well.

Custom Primer	Volume (µl)	Well
Index 1	19	I1
Index 2	19	I2
Read 1	32.4	R1
Read 2	19	R2

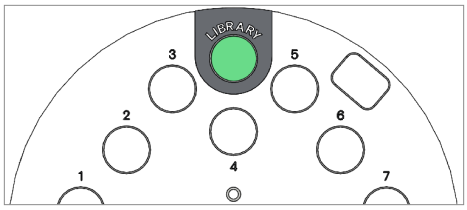
—The final concentration of each custom primer is 1 µM.—

5. Pipette the content of each tube 15 times to mix. Avoid losing existing primer volume.

Add Library to the Cartridge

1. Using a new 1 ml pipette tip, pierce the center of the Library well to create one hole. Push the foil to the edges.

Figure 15: Library well location



2. Discard the pipette tip.
3. Briefly centrifuge the diluted sequencing library to remove bubbles and foam from the tube lid.
4. Transfer the entire volume of diluted sequencing library to the Library well, dispensing along the well wall.
   - » Avoid aspirating any foam or dispensing air.
   - » Do not allow the library to contact the foil.
   - » Make sure the tube contains ≥ 1.3 ml diluted sequencing library.
5. Inspect the library through the window at the front of the basket.
   - » Make sure the library is free of foam and that bubbles are minimal.
   - » If an air gap appears below the surface, use a new pipette tip to remove it.
6. Twist each shipping lock left to unlock.
7. Remove both shipping locks from the cartridge lid.

8. [Optional] Set aside one shipping lock for use during reagent disposal.

Select a Library Prep Workflow

1. Select a library prep workflow:
   - » **Adept**—Sequence libraries prepared with the Adept Workflow.
   - » **Elevate**—Sequence libraries prepared with Elevate indexes and adapters.
2. If you selected Adept, select the **Swap primer tubes** checkbox to confirm that the I1, I2, R1, and R2 wells contain Adept primers or custom primers.
3. Select the **Invert cartridge** checkbox to confirm that reagents are mixed.
4. Select the **Load library pool** checkbox to confirm that the Library well contains diluted sequencing library.
5. Select **Next** to proceed to the Load Reagents screen.

Load Reagents and Buffer

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Slide the basket containing the thawed cartridge into the reagent bay until it stops.
4. Support the buffer bottle with both hands and slide it into the reagent bay until it stops.
5. Close the reagent bay door.
6. Select **Next** to proceed to the Run Side A or Run Side B screen.

Define Run Parameters

1. In the Run Name field, enter a unique name to identify the run.
   —The field accepts 1–64 alphanumeric characters, hyphens (-), and underscores (_).—
2. If applicable, import the run manifest:
   a. Select **Browse**, and then browse to the run manifest for the current run.
   b. Select the run manifest, and then select **Download**.
3. Select a Scan Area option:

Option	Approximate Output*
Full Scan (default)	1 billion reads
3/4	750 million reads
1/2	500 million reads
1/4	250 million reads
1/8	125 million reads

* Read count based on Element control library sequencing. Actual read count might differ based on factors such as library type and preparation.

4. In the Storage list, select a storage location:

» To output run data to the default storage location, leave the default selection.

» To override the default storage location for the current run, select a storage connection.

5. [Optional] In the Description field, enter a description that represents the run.

—The field accepts ≤ 500 alphanumeric characters, hyphens, underscores, spaces, and periods (.).—

6. In the Cycles fields, enter the number of cycles to perform in each read. Add one cycle to the desired number of Read 1 and Read 2 cycles for bioinformatics purposes.

Workflow	Kit	Valid Values				Default Values			
		Index 1	Index 2	Read 1	Read 2	Index 1	Index 2	Read 1	Read 2
Adept v1, v1.1	2 x 75	0–75	0–75	5–184	0–179	Blank	Blank	76	76
	2 x 150	0–75	0–75	5–334	0–329	Blank	Blank	151	151
Adept with Cloudbreak	2 x 75	0–32	0–32	5–184	0–179	Blank	Blank	76	76
	2 x 150	0–32	0–32	5–334	0–329	Blank	Blank	151	151
Elevate v1	2 x 75	0–75	0–75	5–184	0–179	12	9	76	76
	2 x 150	0–75	0–75	5–334	0–329	12	9	151	151
Elevate with Cloudbreak	2 x 75	4–32	0–32	5–180	0–175	12	9	76	76
	2 x 150	4–32	0–32	5–330	0–325	12	9	151	151

—For example, enter 151 in the Read 1 field to perform 150 cycles in Read 1. Entering zero cycles skips the read.—

7. Select **Next** to proceed to the Run Side B or Empty Waste screen.
8. If applicable, repeat steps 1–7 to set up a dual start run.

Empty Waste and Prime Reagents

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
   a. Position the bottle over the funnel or waste receptacle.
      - If you inserted a funnel, align the handle to the inner edge of the funnel.
      - If you did not insert a funnel, center the handle over the waste receptacle.
   b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
   c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to proceed to the Priming screen and ***automatically*** start priming.

—Priming pierces the cartridge seals, so the cartridge cannot be used for another run.—

10. During priming, which takes ~14 minutes, bring a new flow cell to room temperature:

a. Remove a flow cell pouch from 2°C to 8°C storage. ***Do not open the pouch.***
b. Set aside the pouch for ≥ 5 minutes.

11. When priming is complete, select **Next** to proceed to the Load Flow Cell screen.
—AVITI OS moves the nest forward and opens the nest bay door. A brief delay is normal.—

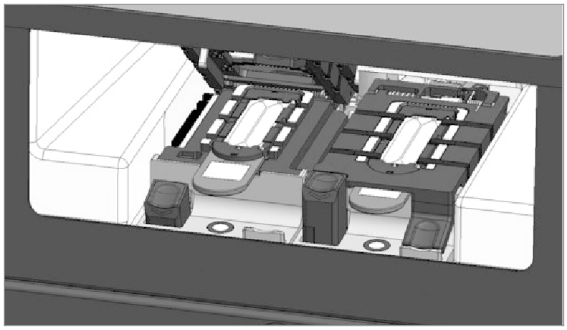
Load the Flow Cell

1. Make sure the nest status light is blue.
2. Press the button to the left of the nest to open the lid.
3. Remove the used flow cell from the nest.
4. Discard the used flow cell or store at room temperature to use for priming and washes.
5. Unpackage the new flow cell. Handle the flow cell by the gripper only.

CAUTION
Touching the glass can introduce debris, smudges, and scratches, compromising data quality.

6. Face the label up and place the flow cell over the three registration pins on the nest.

Figure 16: Loaded flow cells



7. Lower the tab on the right side of the lid until the lid snaps into place.
—The nest status light turns green.—
8. Select **Close Nest** to close the nest bay door and retract the stage.
9. Select **Next** to proceed to the Run Summary screen.

Review and Start the Run

1. Review the run parameters:

Parameter	Description
Workflow	The workflow that prepared the libraries
Kit Size	The supported read length for the sequencing cartridge
Chemistry	The version of the sequencing cartridge and flow cell
Storage	The location where sequencing data are output
Scan Area	The area of the flow cell that the system scans
Cycles	The number of cycles in each read
Description	An optional description of the run

2. Review the flow cell, cartridge, and buffer bottle information:

Field	Description
Lot Number	The number assigned to the batch the consumable was manufactured with
Expires on	The year, month, and date that the consumable expires
Serial Number	The unique identifier for the consumable or all zeros indicating an unscanned barcode
Part Number	The Element-assigned identifier for the consumable

—A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.—

3. Select **Run** to start sequencing.
4. [Optional] If you imported run manifests from a USB drive, disconnect the USB drive:
   a. In the taskbar, select **USB Drive**, and then select **Eject**.
   b. Detach the USB drive from the instrument.
5. Process the materials removed from the reagent bay:
   » If you removed a used cartridge and buffer bottle, follow the instructions in *Discard the Cartridge and Bottle* on page 55.
   » If you removed a wash tray, follow the guidelines in *Wash Tray Maintenance* on page 59.

   —Residual wash solution in the wash tray is normal.—

Monitor Run Metrics

1. If necessary, select **Details** to open run details.
2. Monitor run metrics as they appear onscreen:
   a. After Index 1, cycle 5 for the Elevate Workflow, review Reads and Yield.

b. Within 10 minutes of the index cycles completing, select the **Indexing Stats** tab to review Assigned, Perfect Match, and Samples with Low Representation.

c. After Read 1, cycle 1 for the Adept Workflow, review Reads and Yield.

d. After Read 1, cycle 10, review Q30 on the Home screen. Select the **% Q30** tab to see cycle-by-cycle details.

e. After Read 1, cycle 25, select the **PhiX Error** tab to see the error rates for a run that includes a spike-in.

—All cycles are approximate and all metrics are estimates. Bases2Fastq generates the final metrics.—

3. Continue monitoring the run as AVITI OS refreshes the metrics.

» Each cycle refreshes the Q30 scores, error rates, and index metrics.

» AVITI OS refreshes the yield and reads metrics after cycle 15 of Read 2:

◦ If Read 2 contains no cycles, the refresh occurs after cycle 15 of Read 1.

◦ If Read 1 or Read 2 contain < 15 cycles, the refresh occurs when the last cycle of the read starts.

4. If a run needs to be stopped, see *Stop an Active Run* on page 85.

5. When the run is complete, leave all materials in the instrument.

» To return to the Details view, select **History**.

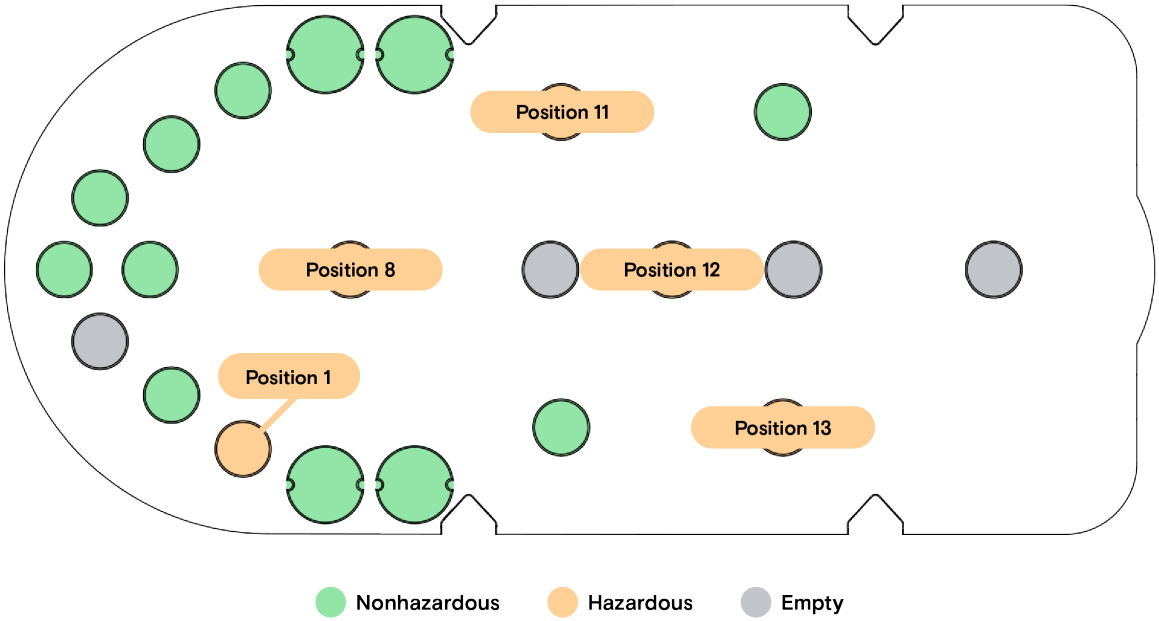
» To access run data, go to your storage location.

Discard the Cartridge and Bottle

The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the SDS at go.elembio.link/sds. The amount of reagent remaining in each well of the cartridge after a run depends on how many cycles the run performed.

The following figure identifies the hazardous reagents in a Cloudbreak cartridge. The reagent position numbers in the figure align with the position numbers in the SDS.

Figure 17: Cloudbreak cartridge map (part # 820-00006, 820-00007)



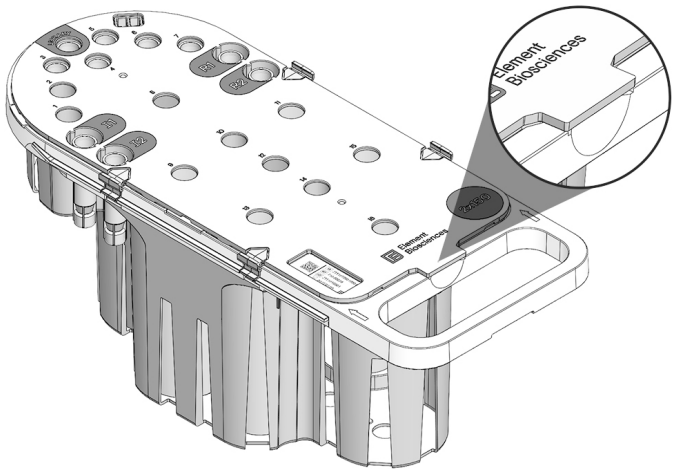
Dispose of Version 1 Reagents

1. Unlock the four clips on top of the basket.
2. Remove the cartridge from the basket.
   —The tabs and divots at each end facilitate removal.—
3. Discard the cartridge and buffer bottle per the SDS.
   —The volume remaining in each depends on the number of cycles performed.—
4. Rinse the basket with nuclease-free water and dry upside down.

Dispose of Cloudbreak Reagents

1. Keep the cartridge in the basket with the clips locked.
2. Grip the lid tab and ***quickly and forcefully*** pull off the lid. Expect resistance.

Figure 18: Lid tab location



3. Remove the wells marked hazardous from the cartridge.

   —The volume remaining in each well depends on the number of cycles performed.—

4. Using the nub on the bottom of a shipping lock or similar tool, enlarge the hole in each foil seal to form a triangle.

Figure 19: Triangular hole in a foil seal

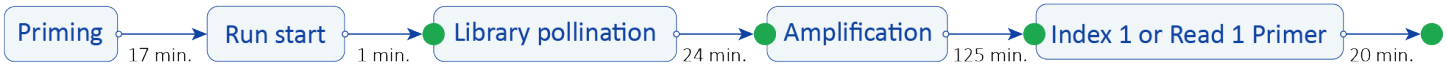
   —The triangular shape facilitates pouring.—

5. Empty each well into hazardous waste or other appropriate container per the SDS.
6. Unlock the clips and remove the cartridge from the basket.
7. Remove the remaining wells from the cartridge.
8. Enlarge the hole in each foil seal as described in step 4.
9. Empty each well into the appropriate container per the SDS.
10. Discard the cartridge and buffer bottle per the SDS.
11. Rinse the basket with nuclease-free water and dry upside down.

Initiate Flexible Start

1. On the Home screen, select **New Run**.
2. When prompted to request flexible start and pause the active run, select **New Run**. Reference the green dots in the following figure to determine optimal pause points with minimal downtime.

Pollination and Amplification



Cycling

Paired-End Turn

Index Priming

—Pausing typically takes several minutes but can take as long as ~2 hours. Durations for the depicted run stages are approximate.—

3. Wait for the run to pause. To cancel flexible start while waiting, select **Cancel Request**.
4. When the run pauses, proceed through setting up and starting the second run. To cancel setup:
   a. Select **Back** to return to the Home screen, and then select **Resume**.
   b. When prompted to confirm that you want to resume the active run, select **Resume**.

—After you start or cancel the second run, AVITI OS resumes the active run.—

CHAPTER 7

Maintenance

Maintenance Schedule

Element recommends the following maintenance schedule to keep the AVITI System in optimal condition and help maintain performance. Every 7 days, AVITI OS warns you that a maintenance wash is due. AVITI OS repeats the warning every 2 days until you perform the wash.

Procedure	Frequency	Purpose
Power cycle	Weekly	Reinitializes the system and resets the instrument computer, which helps maintain instrument performance.
Maintenance wash*	Weekly	Cleans the outside of the sippers and prevents microbial growth and particulate debris from accumulating in the fluidic system.
Standby wash*	Preparing for an idle period of ≥ 7 days	Prepares one or both sides for an idle period of ≥ 7 days.
Air filter replacement	Every 6–12 months	Ensures proper cooling and continuous operation. The optimum frequency depends on lab cleanliness.

* For information on a third wash type, recovery, see *Stop an Active Run* on page 85.

Exterior Cleaning

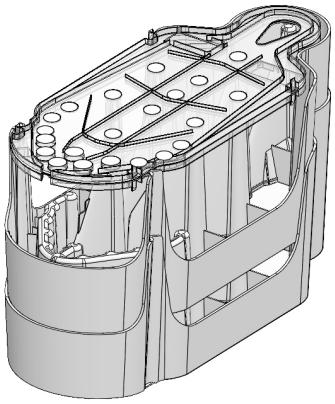
Clean the instrument exterior as needed by wiping the shells with a damp microfiber cloth and using Simple Green to remove oils and fingerprints. Avoid harsh chemicals, cleaners, and abrasives.

Wash Tray Maintenance

The following guidelines for wash tray maintenance keep the wash trays in good condition, maximizing time between replacements and preventing cross-contamination.

- When transporting a wash tray, optionally cover the wash tray with a lid to help prevent spills. Otherwise, a lid is not required.
  - » Keep the lids that come with the gray wash trays with the gray wash trays.
  - » Keep the lids that come with the white wash trays with the white wash trays.
- After each use, discard residual wash solution, rinse the wash tray with nuclease-free water, and air-dry upside down. Rinse the lid with nuclease-free water and air-dry.
- Store clean, dry wash trays upside down with the lids on top of the inverted trays. Stack up to two wash trays with two lids.

Figure 20: Stored wash trays



Warranties and Services

The purchase of an Element AVITI System includes a standard one-year warranty, installation procedures, operational procedures, a performance run, and applications training. For an additional cost, Element offers supplemental procedures, preventative maintenance services, and extended warranties.

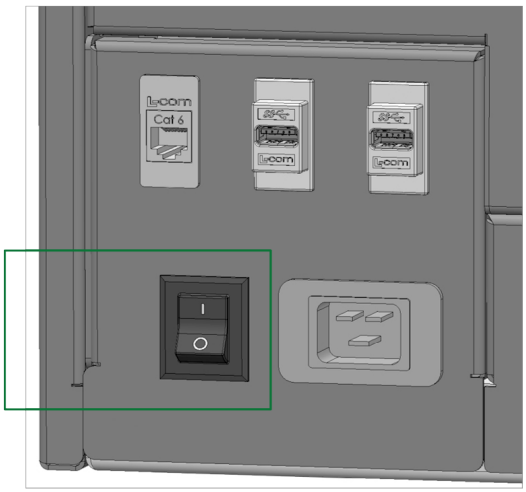
NOTE
Element is both the manufacturer of the AVITI System and the only authorized service provider.

Power Cycle the System

A power cycle resets the instrument computer, safely shutting down and restarting the system to maintain performance or recover from a problem. Turning off the system without a proper power cycle is reserved for emergencies.

1. Select the user menu, and then select **Shut Down**.
2. When prompted, select **Shut Down** again to shut down the instrument computer.
3. Wait a few seconds for the screen to go blank.
4. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.

Figure 21: Power toggle switch in the on position



5. Wait *10 seconds* to make sure the system fully shuts down.
6. On the IO panel, press the power toggle switch to turn on the instrument.

   —The system initializes and displays the Home screen.—
7. If a USB drive is connected to the instrument, reconnect it:

   a. In the taskbar, select **USB Drive**, and then select **Eject**.
   b. Detach the USB drive from the instrument.
   c. Reconnect the USB drive to the instrument.

   —Reconnecting the USB drive allows AVITI OS to detect it after a power cycle.—

Perform a Maintenance Wash

The maintenance wash is a two-part wash that takes a total of ~1.5 hours. Wash 1 cleans the system, removing residual library and carryover. Wash 2 rinses the system, removing residual Wash 1 solution and preparing for the next run. Each wash requires specific volumes of freshly prepared wash solutions.

Prepare Wash Solutions

1. Gather the following materials:
   - » 2 L bottles (2)
   - » 4.00–4.99% sodium hypochlorite
   - » Gray wash tray
   - » Nuclease-free water
   - » Pipette controller
   - » Serological pipettes (2)
   - » Tween 20
   - » Used flow cell
   - » White wash tray

   —A used flow cell might already be in the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 37.5 ml 4.00–4.99% sodium hypochlorite to the bottle to prepare 1.54 L ~0.12% sodium hypochlorite.
5. Label the bottle **Wash 1 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 1 Solution at room temperature. Use within the day or discard.
8. Add 1.5 L nuclease-free water to a new 2 L bottle.
9. Attach a new serological pipette to the pipette controller.
10. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
11. Label the bottle **Wash 2 Solution**.
12. Cap the bottle and invert several times to mix.
13. Set aside Wash 2 Solution at room temperature.

Initiate a Maintenance Wash

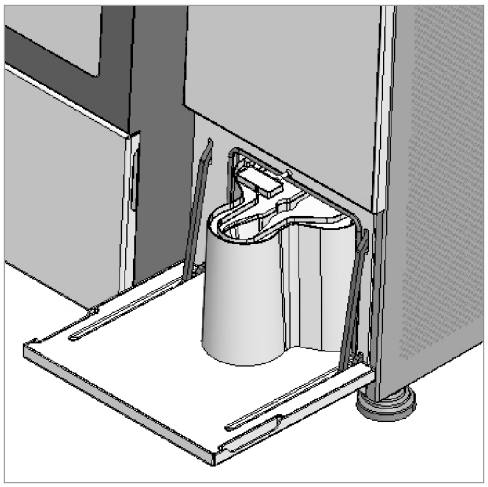
1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a ***used*** flow cell:
   a. Select **Open Nest**.
   b. Place the used flow cell onto the nest and close the lid.
   c. Select **Close Nest**.
3. Select which side to wash:
   - » **Side A**—Set up a maintenance wash on side A.

- » **Both**—Set up maintenance washes on sides A and B.
- » **Side B**—Set up a maintenance wash on side B.

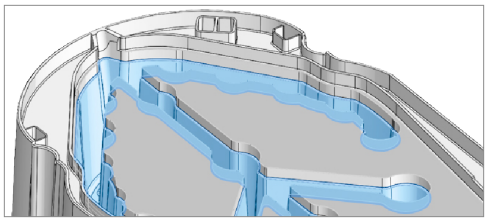
4. Select **Wash**, and then select **Maintenance**.
5. Select **Next** to proceed to the Load Wash 1 screen.

Load Wash 1 Solution

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered gray wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.



5. Add 590 ml freshly prepared Wash 1 Solution to the fill area, filling the wash tray to slightly above the lower fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. Select **Next** to proceed to the Empty Waste screen.

Empty Waste and Run Wash 1

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:

a. Position the bottle over the funnel or waste receptacle.
   - If you inserted a funnel, align the handle to the inner edge of the funnel.
   - If you did not insert a funnel, center the handle over the waste receptacle.

b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.

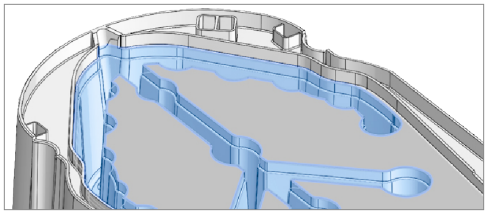
c. If necessary, wipe liquid off the bottle.

7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Wash 1 screen and automatically start the wash, which takes ~34 minutes.
10. During the wash, process the materials removed from the reagent bay:
    - » If you removed a used buffer bottle and sequencing basket, follow the instructions in *Discard the Cartridge and Bottle* on page 55.
    - » If you removed a wash tray, follow the guidelines in *Wash Tray Maintenance* on page 59.
11. When the wash is complete, select **Next** to proceed to the Load Wash 2 screen.

Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the gray wash tray from the reagent bay and set aside.

   —Residual wash solution in the wash tray is normal.—
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

Run Wash 2

1. Select **Next** to open the Run Wash 2 screen and automatically start the wash, which takes ~52 minutes.
2. When the wash is complete, select **Done** to return to the Home screen.
3. Leave all materials in the instrument.
4. Process the gray wash tray from the first wash per *Wash Tray Maintenance* on page 59.

Perform a Standby Wash

A standby wash takes ~52 minutes and flushes nuclease-free water through the fluidic system, removing any residual Tween 20. When complete, the washed side is idle. Performing a maintenance wash on the idle side ends the idle period and enables sequencing.

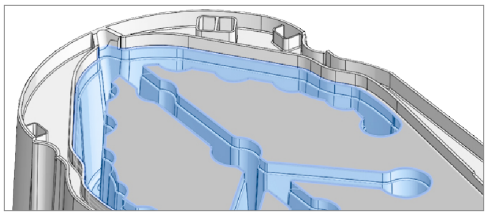
Initiate a Standby Wash

1. Gather the following materials:
   - » Nuclease-free water
   - » Used flow cell
   - » White wash tray

   —A used flow cell might already be in the instrument.—
2. On the Home screen, select **New Run**.
3. If AVITI OS prompts that the flow cell is missing, load a ***used*** flow cell:
   a. Select **Open Nest**.
   b. Place the used flow cell onto the nest and close the lid.
   c. Select **Close Nest**.
4. Select which side to wash:
   - » **Side A**—Set up a standby wash on side A.
   - » **Both**—Set up standby washes on sides A and B.
   - » **Side B**—Set up a standby wash on side B.
5. Select **Wash**, and then select **Standby**.
6. Select **Next** to proceed to the Load Water screen.

Load Nuclease-Free Water

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door.
8. Select **Next** to proceed to the Empty Waste screen.

Empty Waste and Run the Standby Wash

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
   a. Position the bottle over the funnel or waste receptacle.
      - If you inserted a funnel, align the handle to the inner edge of the funnel.
      - If you did not insert a funnel, center the handle over the waste receptacle.
   b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
   c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Water screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Remove Tray screen.

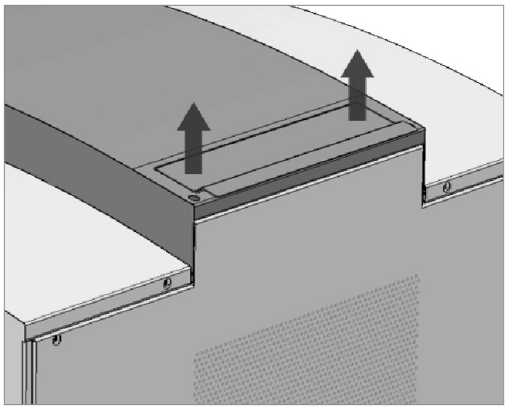
Unload the Wash Tray

1. When prompted, open the reagent bay door and remove the wash tray.
   —Residual water in the wash tray is normal.—
2. Close the reagent bay door.
3. Select **Done** to proceed to the Home screen.
4. Leave the flow cell in the nest.
5. Process the materials removed from the reagent bay:
   » If you removed a used buffer bottle and sequencing basket, follow the instructions in *Discard the Cartridge and Bottle* on page 55.
   » If you removed a wash tray, follow the guidelines in *Wash Tray Maintenance* on page 59.

Replace the Air Filter

Replacing the air filter ensures proper cooling and continuous operation of the system.

1. If the instrument is sequencing or washing, wait for the run or wash to complete.
2. Select the user menu, and then select **Shut Down**.
3. When prompted, select **Shut Down** again to shut down the instrument computer.
4. Wait a few seconds for the screen to go blank.
5. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.
6. Using the flange toward the back of the instrument, lift the air filter tray out of the top.



7. Remove the air filter from the tray and discard.

   —The filter might be loose in the tray, which is normal.—
8. Place the tray on a table or benchtop.
9. With the small arrow on the side of the filter pointing up, place the filter into the tray.
10. Lower the tray into the instrument. Use the pins to align the tray to the rails and guide entry.
11. On the IO panel, press the power toggle switch to turn on the instrument.

    —The system initializes and displays the Home screen.—

CHAPTER 8

System Configuration

System Connections

The AVITI System uses a combination of network, internet, and storage connections to operate. Each system requires a network connection and at least one storage connection. Cloud storage connections, telemetry, remote software updates, and remote support require an internet connection.

Mode	Network Connection	Internet Connection	Storage Connection
Online	Internet	DHCP or static	Cloud or local
	Local	DHCP or static	Local
Offline	Local	None	Local

System Modes

The system mode determines connection options and settings for exporting log files, password protection, and software updates:

- Online mode connects the system to the internet, which streamlines operations.
- Online local authentication mode operates in online mode but includes local authentication, which avoids network requirements to allowlist Auth0 IP addresses. Only an Element representative can enable this mode.
- Offline mode operates the system without an internet connection. Only an Element representative can enable offline mode.

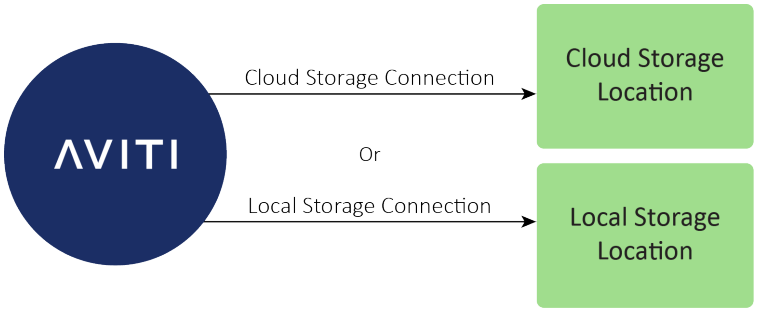
Storage Connections

A storage connection establishes an off-instrument location that AVITI OS transfers files to. Each run delivers bases files and other run outputs to the default storage location unless you specify a different location during run setup.

AVITI OS supports cloud and local storage connections:

- A cloud storage connection transfers files to a storage location in the cloud.
- A local storage connection transfers files to a storage location on a local network or USB drive.

Figure 22: Cloud versus local storage



Storage Connection Requirements

Adding a storage connection requires permissions, network information, and account information that your IT administrator can provide. A cloud storage connection requires an access key and a secret access key, which you can import from a CSV file saved to a USB drive or enter manually.

For comprehensive storage requirements, see the *Element AVITI System Site Prep Guide (MA-00007)*.

Supported Storage Connections

Cloud storage connections include Amazon Web Services (AWS) and Google Cloud Storage (GCS), which is part of the Google suite of cloud computing services. For local storage, AVITI OS supports Server Messenger Block (SMB) and USB.

The storage location for a cloud storage connection is a bucket. A connected bucket is available to all systems. Local storage is exclusive to the system.

Cloud Storage

Cloud Storage Connection	Description
AWS	• Connects the system to an Amazon Simple Storage Service (S3) bucket. • Bases data transfer on secret key authentication through AWS Identity and Access Management (IAM).
GCS	• Connects the system to a Cloud Storage bucket. • Bases data transfer on secret key authentication through a keyed-hash message authentication code (HMAC).

Local Storage

Local Storage Connection	Description
SMB	• Connects the system to the server running SMB via a path to a folder. • Uses the SMB protocol based on service user authentication to transfer data. • Enables import of a run manifest from an SMB storage location during run setup. • Supports automatic export of log files from offline systems. • Supports Kerberos or NTLMv2 authentication.
USB	• Transfers data and log files to a USB drive connected to the instrument. • Supports automatic and manual export of log files from offline systems. • Supports USB-A 3.0 or newer versions and FAT32 or exFAT formats. • Must store a minimum of 1.6 TB of data, which is sufficient for two 2 x 150 runs with indexing.

Configure General Settings

General settings include the instrument name setting and read-only settings that control the instrument profile. For offline systems, general settings also include features to export log files. For instructions, see *Exporting Log Files* on page 79.

Name the Instrument

1. On the taskbar, select **Settings**.
2. Select the **General** tab, and then select **Edit**.
3. Enter a preferred name consisting of 1–20 alphanumeric characters, hyphens (-), and underscores (_) to identify the instrument.

   —The default name is the serial number, field-programmable gate array (FPGA) ID, or Unnamed Instrument.—
4. Select **Save** to apply the name.

Review Read-Only Settings

1. On the taskbar, select **Settings**.
2. Select the **General** tab, and then select **Edit**.
3. Review the following read-only settings. To change a setting, contact Element Technical Support.

Setting	Default	Description
Telemetry	Enabled	Sends instrument health data to Element
High Elevation	Disabled	Calibrates the system to operate at a high elevation
Offline Mode	Disabled	Prevents an internet connection

—Disabling telemetry affects the instrument warranty.—

Connect to the Network

Network settings connect the system to your network via Dynamic Host Configuration Protocol (DHCP) or static IP address. When the system is connected to an Ethernet port, AVITI OS automatically connects to a DHCP server and autopopulates the network settings. Alternatively, you can assign a static IP address and manually configure the network settings.

Select a DHCP Server

1. On the taskbar, select **Settings**.
2. Select the **Network** tab.
3. In the drop-down list, select **Automatic (DHCP)**.

   —AVITI OS assigns a dynamic IP address and all other network settings.—

Assign a Static IP Address

1. On the taskbar, select **Settings**.
2. Select the **Network** tab.
3. In the drop-down list, select **Manual**.

   —AVITI OS assigns a unique and permanent IP address.—
4. Select **Edit**, and then configure the following network settings.

Setting	Example	Description
IP Address	11.2.34.178	The destination IP address
Gateway	11.2.34.177	The IP address of the gateway computer that manages network communications
Subnet Mask	11.2.34.176	The subnet mask that separates the IP address into host and network addresses
Name Server IP(s)	ngs-1.yourlab.com	The names of up to four Domain Name System (DNS) servers that provide IP addresses

   —Two additional network settings, Host name and Mandatory Access Control (MAC) address, are read-only.—
5. Select **Save** to apply the settings and connect to the network.

Add Storage Connections

The Storage tab lists storage connections added to the system, including available storage for each local storage connection. An Element representative adds the first storage connection at installation. After installation, you can add an unlimited number of additional storage connections.

Add an AWS Storage Connection

1. If you are importing the access key and secret access key, prepare the CSV file:
   a. Save the CSV file to a compatible USB drive.
   b. Connect the USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **AWS** as the storage provider.
5. In the Name field, enter a preferred name for the storage connection.
6. Complete the following fields to configure an Amazon S3 bucket as the storage location.

Field	Instruction
Bucket Name	Enter the bucket name, such as NGS-AWS-123.
S3 Prefix	Enter the prefix for the bucket, such as Bucketname/Bucket/January.
Region	Select the region associated with the bucket.

—The Bucket Name and Region fields are required.—

7. Complete the Access Key and Secret Access Key fields using either method:
   » To autopopulate the fields, select **Upload**, browse to the CSV file on the USB drive, select the CSV file, and select **Open**.
   » To manually populate the fields, enter the access key and secret access key.
8. Select **Save** to add the storage connection.
9. If you uploaded a CSV file, disconnect the USB drive:
   a. In the taskbar, select **USB Drive**, and then select **Eject**.
   b. Detach the USB drive from the instrument.

Add a GCS Storage Connection

1. If you are importing the access key and secret access key, prepare the CSV file:
   a. Save the CSV file to a compatible USB drive.
   b. Connect the USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **GCS** as the storage provider.
5. In the Name field, enter a preferred name for the storage connection.

6. Complete the following fields to configure a Cloud Storage bucket as the storage location.

Field	Instruction
Bucket Name	Enter the bucket name, such as NGS-GCS-123.
GCS Prefix	Enter the prefix for the bucket, such as bucket-name.

—Only the Bucket Name field is required.—

7. Complete the Access Key and Secret Access Key fields using either method:
   - » To autopopulate the fields, select **Upload**, browse to the CSV file on the USB drive, select the CSV file, and select **Open**.
   - » To manually populate the fields, enter the access key and secret access key.
8. Select **Save** to add the storage connection.
9. If you uploaded a CSV file, disconnect the USB drive:
   a. In the taskbar, select **USB Drive**, and then select **Eject**.
   b. Detach the USB drive from the instrument.

Add an SMB Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Add Storage**, and then select **Local File Server (SMB)** as the storage provider.
4. In the Name field, enter a preferred name for the storage connection.
5. Complete the following fields to configure an SMB network storage location for the SMB storage connection.

Field	Instruction
Host	Enter the host network IP address or fully qualified domain name (FQDN). The Kerberos authentication protocol requires an FQDN. • Example IP address: 1.222.333.44 • Example FQDN: elembio.com
Port	Enter a port number for the file transfer service or leave blank to accept the default of port 445.
Workgroup/Domain	Enter the name of the work group or domain that the user belongs to. If you are using Kerberos authentication protocol, enter the Kerberos realm name.
Share	Enter the name of the share that makes the directory accessible to SMB.
Path	Enter the path to an ***existing*** folder where you want to output data.
User	Enter the user name for the service user.
Password	Enter the password for the service user.

—All fields except Port and Path are required. Certain server configurations require a work group or domain.—

6. Select **Save** to add the storage connection.

Add a USB Storage Connection

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **USB Drive** as the storage provider.
5. In the USB Drive list, select the USB drive connected to the instrument.
6. In the Name field, enter a preferred name for the storage connection.
7. Select **Save** to add the storage connection.

   —AVITI OS makes sure the USB drive is connected to the instrument and has write permission and sufficient storage.—
8. Disconnect the USB drive:

   a. Select **More** (three dots) for the USB storage connection, and then select **Eject**.

   b. Detach the USB drive from the instrument.

Disconnect a USB for a Storage Connection

1. Select **More** (three dots) for the USB storage connection.
2. Select **Eject**.
3. Detach the USB drive from the instrument.

   —To reuse the USB after disconnecting, reconnect the device to a USB port. The device must maintain the same name for the system to identify the storage connection.—

Manage Storage Connections

Storage settings manage storage connections, including setting the default. Unless you reset the default storage connection, the default is the first cloud location added to the instrument. If a cloud location does not exist, the default storage connection is the first local network location.

You can verify any storage connection, but only local storage connections can be edited and deleted. If you must edit a cloud storage connection, instead create another storage connection.

Verify a Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select the three dots, and then select **Verify Storage**.
4. Wait ~20 seconds for a success message to appear, indicating a valid storage connection.

   —AVITI OS indicates that the connection is connected, unverified, or partially verified with a blocked network.—
5. If AVITI OS cannot verify the storage connection, troubleshoot:

   a. Make sure the storage connection is correctly set up.
      - For an AWS storage connection, check the IAM permissions. See the *Amazon S3 IAM Policy Template* at go.elembio.link/documentation.
      - For a GCS storage connection, check the role assigned to the HMAC key.
      - For an SMB storage connection, check the permissions associated with the users.
      - For a USB storage connection, make sure the USB is not ejected, and check that the USB name and type are correct. For USB requirements, see *Local Storage* on page 70.

   b. If the storage connection is correctly set up, contact Element Technical Support.

Set the Default Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select the three dots, and then select **Set as Default**.
4. When prompted, select **Set Default**.

Edit a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Edit** for the local storage connection you want to update.

   —Editing a busy storage connection can affect where run data are output.—
4. On the Edit Storage Connection screen, edit any of the following fields.

Field	Instruction
Name	Enter a preferred name for the storage connection.
Workgroup/Domain	Enter the name of the work group or domain that the user belongs to. If you are using Kerberos authentication protocol, enter the Kerberos realm name.
User	Enter the user name for the service user.
Password	Enter the password for the service user.

—The Host, Share, Port, and Path fields are read-only. If you must edit these fields, create another storage connection.—

5. Select **Save** to apply the edits and update the storage connection.

Delete a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Delete** for the local storage connection you want to delete.
4. When prompted, select **Delete**.

   —AVITI OS does not allow you to delete a busy storage connection.—

Update the Software

AVITI OS checks online systems for a new software version one time per day. A notification appears when a new version is available and prompts you to perform the update, which occurs remotely and takes ~15 minutes.

For offline systems, Element notifies you of an update and provides the files needed for a manual update. Manual updates are only available for systems in offline mode.

Perform a Remote Update

1. Make sure the AVITI System is not performing a run or wash.
2. On the taskbar, select **Settings**, and then select **Update Software**.
3. When prompted, select **Update Now** to start the update.

   —When the update is complete, the system automatically restarts.—
4. After the system restarts, select **Notifications** to view a notification confirming success.
5. If the update was unsuccessful, contact Element Technical Support.

   —AVITI OS reverts to the previous version so you can continue operation.—

Perform a Manual Update

1. Save the files that Element provides for the update at the root level of a USB drive.
2. Connect the USB drive to a USB port on the side or back of the instrument.
3. Make sure the AVITI System is not performing a run or wash.
4. On the taskbar, select **Settings**.
5. Under Software Update, in the USB Drive list, select the USB drive that contains the update files.
6. When prompted, select **Update Now** to perform the update.

   —When the update is complete, the system automatically restarts.—
7. After the system restarts, select **Notifications** to view a notification confirming success.
8. If the update was unsuccessful, contact Element Technical Support.

   —AVITI OS reverts to the previous version so you can continue using the system.—
9. Disconnect the USB drive:

   a. On the taskbar, select **USB Drive**, and then select **Eject**.

   b. Detach the USB drive from the instrument.

Manage an Offline System

AVITI OS allows you to export log files and password-protect the system. Both features are unique to offline mode and help manage an offline system.

For software update instructions, see *Update the Software* on page 78.

Exporting Log Files

Offline systems support the export of log files using two methods:

- **Automatic export**—Configure AVITI OS to automatically export log files to a local storage location every hour for telemetry purposes. For help connecting exported log files to telemetry, contact Element Technical Support.
- **Manual export**—Export log files to a USB drive as needed to provide troubleshooting resources to Element Technical Support.

By default, automatic export is disabled and AVITI OS does not export any log files. When exporting log files to a USB drive, a solid-state drive (SSD) offers significant time savings compared to a flash drive.

Enable Automatic Export of Log Files

1. If necessary, add a local storage connection to export log files to. For instructions, see *Add Storage Connections* on page 73.
2. On the taskbar, select **Settings**.
3. Select the **General** tab, and then select **Set Up Automatic Export**.
4. In the Storage Connection list, select a local storage connection.
5. Select **Save** to enable automatic export.
6. Transfer the exported log files to an internet-accessible location for telemetry.
7. Delete transferred files from the storage location.

   —Each automatic export adds log files to the storage location without replacing or removing existing files.—

Disable Automatic Export of Log Files

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Disable** to stop automatically exporting log files.

Change the Automatic Export Location

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Edit**.
4. In the Storage list, select a local storage location to export log files to.
5. Select **Save** to reset the location.

Manually Export Log Files

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **General** tab.
4. Under Export Log Files, select **Manual**.
5. In the USB Drive list, select the USB drive connected to the instrument.
6. Select **Export Logs**.

   —AVITI OS exports log files from the last 30 days to the USB drive.—
7. Disconnect the USB drive:

   a. On the taskbar, select **USB Drive**, and then select **Eject**.

   b. Detach the USB drive from the instrument.
8. Upload the log files to the location that Element Technical Support provides.

Manage Passwords

User settings manage passwords for offline systems and online systems with local authentication. Offline systems support setting, changing, resetting, and removing passwords. An online system supports password reset and removal only.

NOTE
Resetting or removing a password requires assistance from Element Technical Support.

Set a Password

1. On the taskbar, select **Settings**.
2. Select the **User** tab.
3. In the Password field, enter a new password.

   —The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
4. In the Confirm Password field, reenter the new password.
5. Select **Save**.
6. When prompted, select **Yes, Set Password**.

Change the Password

1. On the taskbar, select **Settings**.
2. Select **User**.
3. In the Current Password field, enter the current password.
4. In the Password field, enter a new password.

   —The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
5. In the Confirm Password field, reenter the new password.
6. Select **Save** to apply the new password.

Reset a Lost Password

1. On the login screen, select **Forgot Password**.
2. Select **Generate** to display a password reset token and the instrument serial number.
3. Contact Element Technical Support and provide the token and serial number.
   —Element Technical Support emails you a single-use password reset file.—
4. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
5. Connect the USB drive to a USB port on the side or back of the instrument.
6. Select **Next**.
7. Select **Load Reset File** to upload the password reset file, which removes the password from the system.
8. In the Password field, enter a new password.
   —The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
9. In the Confirm Password field, reenter the new password.
10. Select **Reset Password** to apply the new password and return to the login screen.
11. Sign in to the system using the new password.
12. Disconnect the USB drive:
    a. On the taskbar, select **USB Drive**, and then select **Eject**.
    b. Detach the USB drive from the instrument.
13. Discard the password reset file.

Remove the Password

1. On the taskbar, select **Settings**.
2. Select **User**, and then select **Remove Password**.
3. When prompted, select **Yes, Remove Password**.
4. Select **Generate** to display a password reset token and the instrument serial number.
5. Contact Element Technical Support and provide the token and serial number.
   —Element Technical Support emails you a single-use password reset file.—
6. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
7. Connect the USB drive to a USB port on the side or back of the instrument.
8. Select **Next**.
9. Select **Load Reset File** to upload the password file, which removes the password from the system.
10. Disconnect the USB drive:
    a. On the taskbar, select **USB Drive**, and then select **Eject**.
    b. Detach the USB drive from the instrument.
11. Discard the password reset file.

CHAPTER 9

Troubleshooting

General Troubleshooting

Error messages communicate hardware or software problems and describe both the problem and resolution. General troubleshooting resolves other problems that can occur during system initialization, run setup, and sequencing. If a problem persists, contact Element Technical Support.

A power cycle resolves many common problems. For instructions, see *Power Cycle the System* on page 61.

Initialization Problems

Power cycle the system to resolve the following initialization problems:

- After turning on the instrument, the monitor does not display AVITI OS.
- The initialization sequence is incomplete, so the loading screen remains after ~10 minutes.

Run Setup Problems

Problem	Resolution
The flow cell is cracked, scratched, or otherwise damaged.	Contact Element Technical Support.
The lid does not engage when a flow cell is on the nest.	Remove the flow cell and wipe the nest. Inspect the flow cell for large debris and wipe with an alcohol pad if necessary. Reload the flow cell.
AVITI OS detects a full waste bottle, but the bottle is empty.	Reload the waste bottle and make sure the waste bay is unobstructed.
AVITI OS cannot detect a loaded sequencing cartridge or waste bottle.	Follow the onscreen prompt to reload the sequencing cartridge or waste bottle. Make sure the applicable bay, reagent or waste, is unobstructed, and that the sequencing cartridge is contained within a sequencing basket.
The system cannot scan or detect a barcode on the sequencing cartridge, buffer bottle, or flow cell.	Follow the onscreen prompt to reload the consumable or continue without scanning by entering the consumable part number.
The flow cell version is incompatible with the sequencing cartridge.	Load a flow cell that is the same version as the cartridge.
The reagent bay contains condensation.	Dry the inside of the reagent bay with a clean, dry microfiber cloth. Clean to the back of the bay, avoiding sensors and cables.

Problem	Resolution
The reagent or waste bay contains liquid.	See *Clean Spills and Leaks* on page 88.
Liquid is spilling from the front or bottom of the instrument.	
The nest is wet.	

Sequencing Problems

Problem	Resolution
The software, instrument, keyboard, or mouse stopped operating.	Power cycle the system.
Sequencing continues after you stop a run.	Wait for the run to stop. AVITI OS waits for a safe point to stop the run, which can take several minutes to ~2 hours depending on the run stage.
The run folder is missing data.	Make sure the user interface indicates that data are uploading and wait for the upload to complete. • A slow connection delays data transfer. • Data transfer failure prompts a notification.
Polony density is lower or higher than expected.	Contact Element Technical Support or stop the run. See *Stop an Active Run* on page 85.
The assigned or perfect match metrics are lower than expected.	Make sure that the index sequences recorded in the run manifest are correct.
The samples with low representation metric is higher than expected.	Select **Sample Details** to view the samples with low representation. Make sure that the index sequences recorded in the run manifest and the pooling concentration are correct.
The Q30 percentage is lower than expected.	Contact Element Technical Support.
The PhiX error rate is higher than expected.	
The flow cell contains very few polonies or no polonies.	
The user interface is frozen.	

Cancel Runs and Washes

AVITI OS displays the following buttons for canceling runs and washes:

- **Discard**—Appears on every run and wash setup screen and cancels setup. The button is enabled when you can discard the run or wash without compromising the consumables.
- **Stop**—Appears on the Home screen and cancels an active run. The button is always enabled so you can free the instrument when run parameters are incorrect, data quality is poor, or a hardware problem occurs.

Discard Run Setup

1. On any run setup screen before priming, select **Discard**.
2. When prompted to confirm the discard, select an option:
   - » **Unlock Door A** or **Unlock Door B**—Discard the run and save the sequencing cartridge.
   - » **Discard Setup**—Discard the run, delete the run, and return to the Home screen without saving the cartridge.
3. If you unlocked the door, proceed with the remaining steps.
4. Open the reagent bay door and remove the cartridge.
5. Place the cartridge on ice or refrigerate at 2°C to 8°C.
6. Place a clean, uncovered white wash tray onto the open reagent bay door.
7. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
8. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.
9. Slide the wash tray all the way into the reagent bay until it stops.
10. Close the reagent bay door.
    —AVITI OS deletes the run setup and returns to the Home screen.—
11. Set up a new run and use the cartridge within ***4 hours***.

Discard Wash Setup

1. On any wash setup screen, select **Discard**.
2. When prompted to confirm the discard, select **Discard Setup**.
   —AVITI OS deletes the wash setup and returns to the Home screen.—

Stop an Active Run

Stopping an active run is a two-part process: stop the run, then perform a ~60-minute recovery wash to remove residual library from the fluidic system. To start the recovery wash, the other side must be idle.

CAUTION
Stopping a run is ***final***. You cannot resume a stopped run or reuse any of the consumables.

Stop the Run

1. On the applicable side of the Home or Run Details screen, select **Stop**.

2. When prompted, select **Yes, Stop Run**.

   —AVITI OS finishes the current step, terminates the run, and returns to the Home screen.—
3. If the other side is sequencing or washing, wait for the run or wash to complete.
4. Proceed to *Prepare Wash 2 Solution* and complete the recovery wash.

Prepare Wash 2 Solution

1. Gather the following materials:
   - 2 L bottle
   - Nuclease-free water
   - Pipette controller
   - Serological pipette
   - Tween 20
   - Used flow cell
   - White wash tray

   —A used flow cell might already be in the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
5. Label the bottle **Wash 2 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 2 Solution at room temperature.

Initiate a Recovery Wash

1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a ***used*** flow cell:
   a. Select **Open Nest**.
   b. Place the used flow cell onto the nest and close the lid.
   c. Select **Close Nest**.
3. Select which side to wash:
   - **Side A**—Set up a recovery wash on side A.
   - **Both**—Set up recovery washes on sides A and B.
   - **Side B**—Set up a recovery wash on side B.
4. Select **Wash**, and then select **Recovery**.
5. Select **Next** to proceed to the Load Wash 2 screen.

Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the buffer bottle and sequencing basket from the reagent bay. Set aside both materials.

3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.
6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door.
8. Select **Next** to proceed to the Empty Waste screen.
9. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

Empty Waste and Run Wash 2

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
   a. Position the bottle over the funnel or waste receptacle.
      - If you inserted a funnel, align the handle to the inner edge of the funnel.
      - If you did not insert a funnel, center the handle over the waste receptacle.
   b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
   c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Wash 2 screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Remove Tray screen.

Unload the Wash Tray

1. When prompted, open the reagent bay door and remove the wash tray.
   —Residual wash solution in the wash tray is normal.—
2. Close the reagent bay door.
3. Select **Done** to proceed to the Home screen.
4. Leave the flow cell in the nest.
5. Discard the sequencing cartridge and buffer bottle and wash the basket. See *Discard the Cartridge and Bottle* on page 55.

Clean Spills and Leaks

Clean the nest, waste bay, or reagent bay to recover from a leak or spill observed when setting up a run or wash. A leak or spill that occurs in the waste bay during a run causes an error and requires cleaning and contacting Element Technical Support.

If the bottom of the instrument is leaking or liquid is spilling from the front of the instrument: shut down and unplug the instrument if doing so is safe and contact Element Technical Support.

Clean the Nest

1. Dampen a microfiber cloth with isopropyl alcohol.
2. Wipe the nest with the damp microfiber cloth and allow to dry.
3. If necessary, use a polyurethane foam-tip swab to clean additional areas around the nest.
4. Resume run or wash setup.

Clean the Reagent Bay

1. Keep the reagent bay door open.
2. Remove any materials from the reagent bay and set aside.
3. Wipe the interior of the reagent bay with a damp microfiber cloth, cleaning to the back of the bay while avoiding sensors and cables.
4. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
5. Resume run or wash setup.

Clean the Waste Bay

1. Keep the waste bay door open. If the leak occurs during a run, open the door:
   a. Wait for any runs or washes on the unaffected side to finish.
   b. On the taskbar, select **Notifications**.
   c. On the applicable error, select **Unlock Waste Module Door**.
   d. Open the waste bay door.
2. Unscrew the transport cap from the cap holder on the affected side.
3. Remove the waste bottle from the waste bay and close the transport cap.

   CAUTION
   Waste droplets might be on the exterior of the waste bottle.
4. Inspect the waste bottle for cracks, holes, and other defects.
5. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
6. Open the transport cap and the vent cap.
7. Support the waste bottle with both hands and empty the waste:
   a. Position the bottle over the funnel or waste receptacle.
      - If you inserted a funnel, align the handle to the inner edge of the funnel.
      - If you did not insert a funnel, center the handle over the waste receptacle.

b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.

c. If necessary, wipe liquid off the bottle.

8. Close the vent cap, leave the transport cap open, and set aside the waste bottle.
9. Wipe the interior of the waste bay with a damp microfiber cloth.
10. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
11. Return the waste bottle to the waste bay.
    - » If the bottle is defective and you have a spare, load the spare.
    - » If the bottle is defective and you do not have a spare, load the defective bottle. Do not use the affected side until the defective waste bottle is replaced.

    —A run or wash on either side requires the presence of both bottles.—
12. Screw the transport cap onto the cap holder and close the waste bay door.
13. Resume run or wash setup. If necessary, set up a new run with new consumables and clean accessories.

CHAPTER 10

Safety and Compliance

General Safety

Review this chapter before operating or maintaining the Element AVITI System to ensure safe, correct usage. The procedures described in this guide are tested and optimized, so any deviation can compromise results, cause personal injury, or damage the instrument. All personnel operating the instrument must be trained in correct operation and safety.

The *Element AVITI System Site Prep Guide (MA-00007)* provides delivery information and installation requirements, including instrument specifications, power specifications, and environmental conditions. A field service engineer (FSE) installs the AVITI System.

WARNING
Do not attempt to move the instrument, which can result in injury. Only trained Element personnel are qualified to safely move the instrument.

Safety Labels

The following table lists the safety labels affixed to the instrument. The labels identify potential hazards associated with installation, service, and operation. Follow the procedures in this guide as described to avoid interactions that expose you to these hazards.

WARNING
This product can expose you to chemicals including formaldehyde, which is known to the State of California to cause cancer, and methanol, which is known to the State of California to cause birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov.

Potential Hazard	Label	Description
Class 4 Laser		The instrument is a Class 1 laser product that contains a Class 4 laser. See *Laser Safety* on page 92.
Heat hazard		The nest has a hot surface and exposure can cause burns.

Certification Body Mark

In addition to the safety labels, the following Nemko label is affixed to the instrument. The label indicates that the instrument complies with Certification Body requirements in the US and Canada.

Figure 23: Label indicating Certification Body compliance

Laser Safety

The AVITI System is certified as a Class 1 laser product per the US Federal Product Performance Standard for Laser Products requirements described in 21 CFR Subchapter J. The exception to these requirements is the deviations described in FDA Laser Notice #56. The product is classified per IEC/EN 60825-1:2014.

WARNING
Adjusting or performing procedures other than those described in this guide or other Element guides can result in hazardous radiation exposure.

Class 4 Laser

The instrument is a Class 1 laser product that contains a Class 4 laser. The Class 4 laser produces Class 4 levels of visible laser radiation, which can be hazardous to eyes and skin. Protective shells and safety interlocks prevent exposure or access to laser radiation levels that exceed Class 1 during operation, maintenance, or normal service.

The following figure depicts the label that identifies noninterlocked portions of the shells that prevent access to laser radiation. Additionally, the nest bay and both reagent bays contain barcode scanners that emit Class 1 levels of laser radiation.

Figure 24: Label identifying noninterlocked locations

Operating Conditions

Do not operate an AVITI System with bypassed interlocks, damaged shells, or any portion of the shells removed. These conditions make Class 4 levels of laser radiation possible and risk exposure to direct or reflected laser light.

Only Element service personnel, Element-authorized agents, or Element-trained personnel can perform services that require internal interlock bypass or removal of portions of the shells. If you are present during service, take the proper safety precautions to mitigate the risk of direct and reflected laser light.

Product Compliance

The AVITI System is verified as meeting the following standards:

- FCC 47 CFR Part 15, equipment authorization – radio frequency (RF) device
- IEC 61326-1, EMC/EMI requirement for lab equipment

The instrument is certified to the following additional standards:

- IEC 60825-1, Class 1 laser product classification
- IEC 61010-1, general safety requirements
- IEC 61010-2-010, requirements for lab equipment and heating materials
- IEC 61010-2-081, requirements for automatic and semi-automatic equipment
- UL 61010-1, safety requirements for electrical equipment

FCC Compliance

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

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Technical Support

Visit the User Documentation page on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

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Document History

Document #	Date	Description of Change
Document # MA-00008 Rev. D	April 2023	• Updated software descriptions to AVITI OS v2.0.0. • Updated instructions on custom primers, run setup, reagent disposal, discarding runs, storage connections, and exporting log files. • Updated run statistic population times and added index assignment. • Updated lightbar colors to include washes, warnings, and errors. • Updated descriptions of the Home screen, run stages, settings, telemetry, run manifest, storage connections, and storage locations. • Updated the links for accessing user guides, templates, and safety data sheets. • Added a chemical exposure warning for Proposition 65. • Added an expected wait time for flexible start. • Added custom primer requirements. • Added instructions and a notification for software updates. • Added network and storage status indicators. • Added a USB storage connection and taskbar icon. • Added the High Elevation setting and removed the Dark Mode setting. • Added Element oligonucleotide contents. • Added troubleshooting for barcode scanning, flow cell compatibility, and index assignment. • Added LoopSeq for AVITI as a compatible library and kit compatibility. • Added the following Element products: AVITI 2x75 Sequencing Kit Cloudbreak (catalog # 860-00004), AVITI 2x150 Sequencing Kit Cloudbreak (catalog # 860-00003), Adept Custom Primer Set (catalog # 820-00009), and Cloudbreak PhiX Control Library, Elevate (catalog # 830-00017). • Identified the reagents in each well of a Cloudbreak cartridge. • Consolidated instructions on replacing primers, denaturing and diluting libraries, and cleaning the waste bay. • Replaced run.prodstats with AvitiRunStats.json. • Recommended a weekly power cycle. • Moved power cycle instructions from troubleshooting to maintenance. • Renamed AOS to AVITI OS and run statistics to run metrics. • Renamed AOS to AVITI OS, run statistics to run metrics, and the Workgroup field to Workgroup/Domain.

Document #	Date	Description of Change
Document # MA-00008 Rev. C	October 2022	• Updated software descriptions to AOS v1.2.0. • Updated run statistic population times. • Updated the read counts for approximate run output. • Updated the internet connection for local online networks to DHCP or static. • Updated navigation for the Home, Notifications, and Settings workspaces. • Updated loading concentrations for Adept libraries. • Updated spike-in recommendations for PhiX Control Library. • Updated the buffer bottle design. • Updated instrument certifications and laser labeling. • Updated trademark and patent information in the legal notice. • Renamed the Error Rate tab to PhiX Error. • Renamed the Element Adept Library Compatibility Kit to Element Adept Library Compatibility Kit v1.1. • Renamed the AVITI Sequencing Kit to AVITI 2x150 Sequencing Kit. • Added Element catalog # 860-00002 for the AVITI 2x75 Sequencing Kit. • Added Element catalog # 820-00008 for the Adept Custom Oligonucleotide Buffer Set. • Added custom primer information and instructions. • Added thaw times for the 2 x 75 sequencing cartridge. • Added error handling for exceeding the maximum number of cycles. • Divided the instructions to mix reagents and add library into two procedures. • Specified that primary analysis must be complete before setting up a run. • Replaced Windex glass cleaner with Simple Green All-Purpose Cleaner. • Marked the FAT32 USB drive as optional. • Corrected the list of library dilution consumables.

Document #	Date	Description of Change
Document # MA-00008 Rev. B	July 2022	• Updated software descriptions to AOS v1.1.0. • Updated reagent thawing, power cycling, and logoff instructions. • Updated run folder content: added statistics and upload files, removed log files, and updated bases file extensions. • Added requirement to protect the cartridge from light throughout storage, preparation, and run setup. • Added a GCS storage connection and an offline mode. • Added lightbar colors that indicate system status. • Added an onscreen keyboard and removed the requirement to connect a keyboard and mouse. • Removed the option to view notifications when signed out. • Renamed the settings chapter to *System Configuration* and reorganized. • Clarified the work group definition and requirement for an SMB storage connection. • Decreased the priming duration and increased maintenance, standby, and recovery wash durations. • Consolidated information on run and wash setup screens.
Document # MA-00008 Rev. A	June 2022	• Initial release

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